proportion of stems inclining towards the E was higher than the proportion of stems inclined towards the W. At the end of the process, the proportion of stems inclined towards each inter-row became similar because the proportion of plants inclined towards the W increased. At the highest crop population density (14 plants m^{-2}), the stem inclination process began 15-20 dae, before the beginning of floral initiation. A large (≈70%) proportion of the stems showed inclination at the time when floral initiation was completed in these plots (Fig. 1).

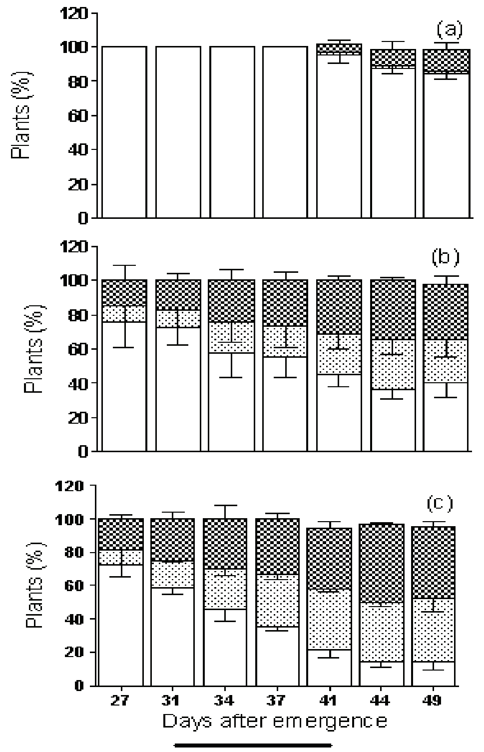

Fig. 1. Dynamics of stem inclination from 20 days after emergence to bud visible in crops sown at three crop population densities: a) 5 plants m^{-2}, b) 10 plants m^{-2}, and c) 14 plants m^{-2}. Each bar represents the proportion of plants not inclined (white), inclined towards the E (heavy stippling) and towards the W (light stippling). The line below panel c) represents the duration of the floral initiation period in these crops. The vertical lines on the bars indicate ± a standard error, n= 3 (40 plants per replication). Experiment 1.

In Experiment 2, stem inclination began shortly after neighbouring plants began to be shaded by their neighbours (Fig. 2), with the shaded plant inclining away from the row axis towards the inter-row space. The plants that first experienced shading were the first to show this response (e.g., Plants 1 and 2 vs. Plant 3, Fig. 2). The effects found in the 14 remaining plants evaluated in this experiment (data not shown) were consistent with those illustrated for the three plants shown in Fig. 2.

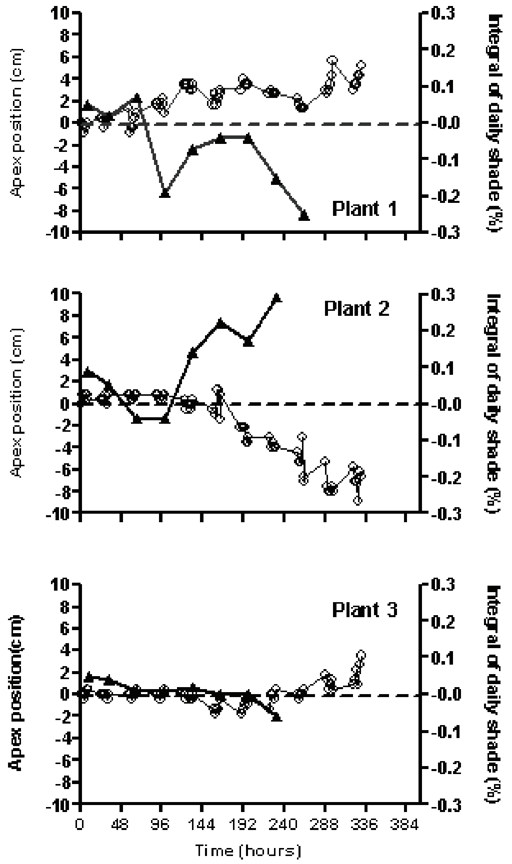

Fig. 2. Dynamics of the apex position (cm from row axis, circles, five observations per day) and daily shade integral (mean daily fraction of total plant leaf area, triangles) for three plants growing in plots sown at 20 plants m^{-2}. The dashed horizontal line indicates the row axis. The negative and positive values represent the two inter-rows, and are used to show the position where the shading took place and position of the plant apex. Daily shade integral estimates were not made after about 280 hours after the start of observations (8 dae) because the degree of plant-to-plant interference became too difficult to resolve in the two-dimensional images.

In Experiment 3, proportion of inclined plants and the angle of stem inclination evoked by low R/FR incident on the upper leaves were greater than in the other two treatments (little response in low blue light treatment and insignificant for the control (neutral filter) treatment) (Fig. 3). Clearly, light of a low R/FR ratio incident on the leaves can evoke stem inclination. The R/FR ratio measured below the upper leaves close to the row axis was 0.55±0.12, in contrast to 1.17±0.07 above the upper leaves close to the inter-rows. These values are consistent with those obtained using the R/FR filters.

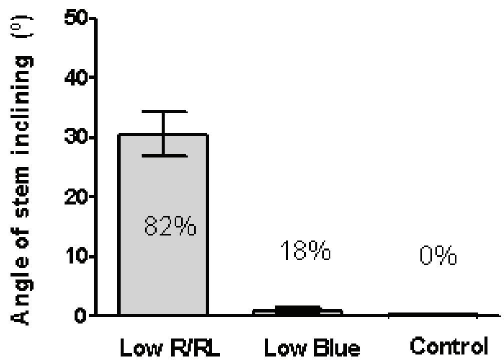

Fig. 3. Effects of light of low red/far red (R/FR) ratio, low blue (B) and normal (sunlight) R/FR and blue (control) on the angle of inclination of sunflower stems. The numerical values on or above each column indicate the proportion (%) of inclined plants (n=10) in each treatment. The vertical lines on the bars indicate ± a standard error. Experiment 3.

In Experiments 4 and 5, grain yield was significantly (Exp.4 $p \leq 0.03$, Exp.5 $p \leq 0.01$) lower in plots in which stem inclination was forcibly restricted (Exp.5 = 25.2 ±1.5; Exp. 6 =16.9 ± 1.8 g per plant) than in plots in which the natural self-organization was allowed (Exp.5 = 30.6 ±0.7; Exp. 6 =31.4 ±0.7 g per plant). These effects on grain yield were associated with significant reductions in grain number per plant in both experiments (Exp.4 $p \leq 0.01$; Exp.5 $p \leq 0.01$).

DISCUSSION

Our results indicate that the process of crop self-organization is an important response of sunflower crop to high crop population densities. This process occurs over time, is hastened at high crop population densities and the propagation of the pattern occurs in patches. Mutual shading between leaves of neighbouring plants (briefly) precedes the deviation of the stems from the vertical. The fact that low R/FR ratios in the light incident on the upper leaves can evoke stem inclination is consistent with the reduction in R/FR ratio of light transmitted through green leaves. The weak response to low blue light of the process (light transmitted through leaves is depleted in blue) suggests that phytochromes rather than cryptochromes or phototropins are the photoreceptors involved. This is the first time that it has been shown that the quality of light impinging on leaves can alter stem position (previous reports in other species involve detection/response associations limited to either leaves (Maddoni et al., 2002) or stems (Ballaré et al., 1991) The process would appear to have an effect on crop yield. It remains to be established whether the effect on crop yield is mediated by changes in resource capture (e.g., higher fractional interception); resource allocation (e.g., less competition for biomass between stem and floral structures); direct photomorphogenic effects (e.g., in soybean, the abscission of reproductive structures in the lower strata of the canopy during flowering was lower in plots with high R/FR; Heindel and Brun, 1983); or a combination of these effects.

Another issue arising from the identification of this process relates to possible intra-specific variability for the intensity of this process (and we have preliminary evidence to support this possibility). In Argentina, there is some interest in intersown sunflower/soybean crops. An important issue here is the conditions for soybean seedling establishment and early growth in the space between the rows of sunflower. Sunflower hybrids less sensitive to signals produced by neighbouring plants might produce less shade for the soybean intercrop. By contrast, in non-uniform stands of pure sunflower crops (a common circumstance in sunflower crops in Argentina), it would be desirable to have cultivars capable of responding to environmental heterogeneity and ensuring greater resource capture.

ACKNOWLEDGEMENTS

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Early sowing as a means of drought escape in sunflower: effects on vegetative and reproductive stages

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ABSTRACT
Drought occurring mainly during the flowering period is responsible for a substantial decrease in the production in sunflower. The hypothesis that early sowing of sunflower would increase the probabilities of avoiding warm and dry period during the reproductive stage was tested. Nevertheless, early sowing is associated with low temperature during the first development stages. Phenological determinations were performed to study the effect of early sowing on the vegetative and reproductive periods. A sunflower population of recombinant inbred lines (RILs) was studied in two sites (France and Algeria) during 2007 at normal sowing date (control) and one-month earlier sowing. Phenostage observations were determined from emergence to harvest. Weather data with rainfall and temperatures were daily recorded. Cumulative growing degree day requirements for each phenological stage were calculated. Earliness of flowering was observed in the two sites when sunflower genotypes were sown one-month earlier in the season. Differences in thermal time requirement for sunflower development observed between sites and between early and control sowings could be explained by variations in base temperature values and/or photoperiod effect. A significant variability between genotypes was observed for sunflower development. The genotype ranking was not affected by early sowing for vegetative stage on the two sites, but during the post-flowering stage in Algeria, high temperatures and dry conditions occurring during this period considerably reduced their variability in the phenostage (R6-R9) and modified the genotype ranking. Genetic basis of sunflower phenostages response to early sowing must be explored in terms of genetic variability for temperature x photoperiod interactions.

Key words: drought escape – early sowing – *Helianthus annuus* L. – low temperatures – phenological stage – sunflower.

INTRODUCTION
Two main strategies are considered to increase sunflower productivity of non irrigated cropping systems. The first consists of selecting genotypes tolerant to dehydration during the water deficit conditions. Poormohammad Kiani et al. (2007a, b) have studied physiological traits of sunflower under drought conditions and differential expression of water stress-associated genes in order to supply tools for drought tolerance selection. Another way is to modify cultural practices to avoid drought at flowering stage. In French cropping systems, most farmers sow around 15 April and flowering takes place under high evaporative demand and scant rainfall. Consequently, the crop is often subject to water deficit and yield decrease. A possible alternative strategy for avoiding drought at flowering is to sow earlier, at times of lower evaporative demand. Early sowing including winter planting was tested in several Mediterranean countries (Gimeno et al., 1989; De La Vega et al., 2002; Flagella et al., 2002). It was shown that this approach allowed to increase water availability (Barros et al. 2004; Soriano et al. 2004). Therefore, the yield was increased (Hadjichrisyodoulou et al., 1987; Gimeno et al., 1989; Tenteiro et al. 1994; Anastasi et al. 2000). However, a major disadvantage of growing crops during low-evaporative-demand periods is the common association between low evaporation and low temperature.
The aim of the present work was to determine the effect of early sowing on vegetative and reproductive stages in a population of 100 recombinant inbred lines (RILs) of sunflower with a large genetic variability, through two experiments conducted in contrasted pedoclimatic conditions (France and Algeria).

MATERIALS AND METHODS

Plant material and field experiments

A population of 98 RILs of sunflower (*Helianthus annuus*) and their parents RHA 266 and PAC2 (Flores Berrios et al., 2000; Poormohammad Kiani et al., 2007a) were used to investigate early sowing in term of vegetative and reproductive stages. Genotypes were tested in two locations: in France (Toulouse: 43°31’46,94” N; 1°29’59,71” E) and in Algeria (Constantine: 36°16’17.65”N; 6°40’13.01”E). In France, field experimentation was conducted at INRA station of Auzeville, and in Algeria at CNCS station of El-Khroub. For the last ten years, the French site had low temperatures in winter and the Algerian site had warm and dry conditions during summer (Fig. 1). Weather data were obtained from Meteo France.

In 2007, RIL population was sown on two dates in both sites: 14 March (early sowing: ES-F) and 19 April (control sowing:CS-F) in France and 3 March (early sowing ES-A) and 26 March (control sowing: CS-A) in Algeria. Three replications by sowing date were performed. Each replication consisted of two rows 3m long, with 50cm between rows and 25cm between plants in rows, giving a total number of about 24 plants per experimental unit.

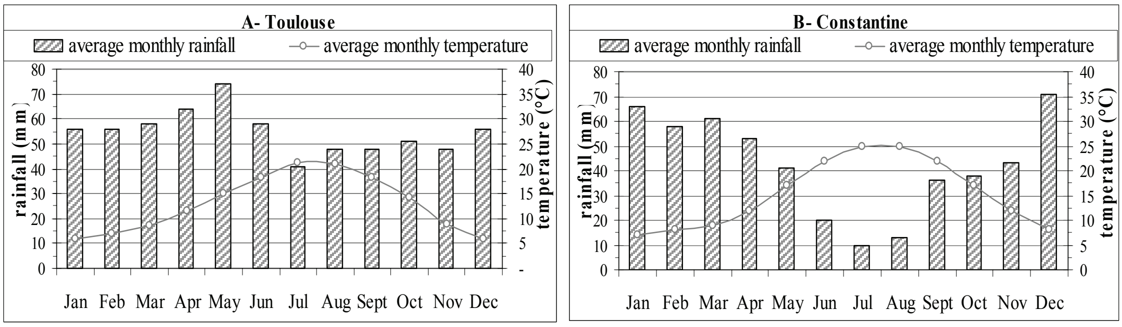

Fig 1. Average monthly temperatures and average monthly rainfalls (mean of the last 10 years) in the two experimental sites (Toulouse in France and Constantine in Algeria).

Phenological measurement

Plant development was recorded according to the definition of growth stage system of Schneiter and Miller (1981). Dates were obtained for 100% emergence (VE), 50% of plants at beginning of flowering (R5), 50% of plants at complete flowering (R6), 50% of plants at physiological maturity (R9) and 50% of plants at harvest. The results were recorded as Vegetative Period (VP) from sowing to R5 and Post-Flowering Period (PFP) from R5 to harvest.

Daily maximum and minimum temperatures and rainfall were recorded at each site. Cumulative growing degree days (°Cd) were calculated as the sum of the average daily temperature minus base temperature of 4.8°C (Granier and Tardieu, 1998).

Statistical analysis

Statistical analyses were performed with SPSS for Window (11.0.1). Sowing date, location and genotype effects were tested using ANOVA procedure. Correlation between control and early sowing for vegetative period (VP) and post-flowering period (PFP) in cumulative growing degree days were performed in France and in Algeria sites. Moreover, correlations between VP and PFP were realized for each sowing date in both sites.

RESULTS

Sunflower development (vegetative and reproductive stages)

The two sites (France and Algeria) presented substantial differences during the growing season: colder at the first phenostages and warmer during post-flowering stage in Algeria (Fig. 2A). Fig. 2B shows that the total of time cycle length was significantly different between the two controls even if differs for only 5 days with 130 days in France and 125 days in Algeria. Vegetative development period represented 62% of total duration in France vs. 72% in Algeria. In fact, time to emergence (sowing-VE) was twice longer in Algeria compared with France. Temperatures corresponding to sowing-VE were inferior in Algeria (13°C) than France (15°C) (Fig. 2A). The time between VE and R5 stage did not differ with, respectively, 73 days and 74 days in France and Algeria. The post-flowering period (PFP) with respect to sowing-harvest duration was proportionally shorter in Algerian location. With the same flowering time (10 days), the period from R6 to harvest differed between the two sites (9 days more for R6-R9 in France, and 5 days more for R9-harvest in Algeria).

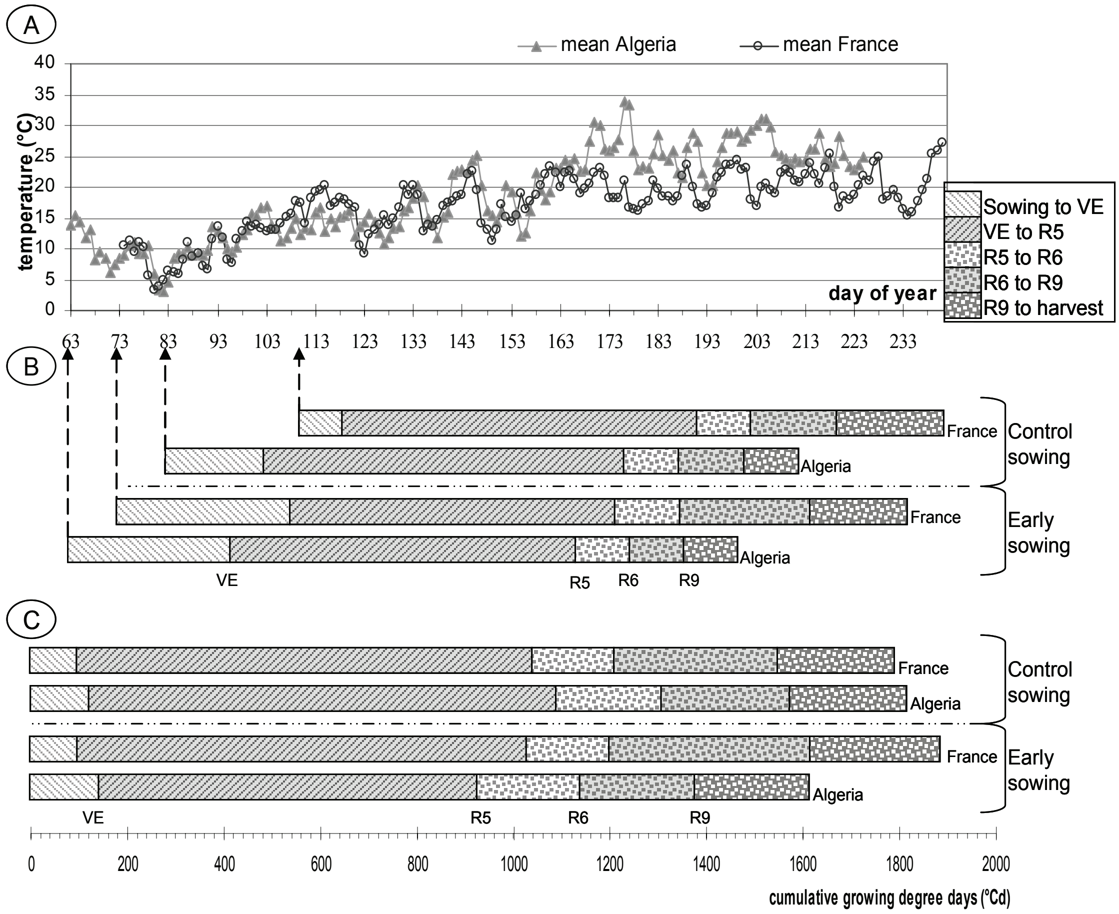

Fig 2. Mean temperature for control and early sowing in France and Algeria (A). Sunflower phenostages in relation to day of year (B) or to cumulative growing degree days with tb = 4.8°C (C).

Whereas total cycle length differed between France and Algeria, there was no significant difference for cumulative growing degree days with, respectively, 1857 and 1871°Cd (Fig. 2C). Cumulative growing degree days required for vegetative period (VP) was higher in Algeria than in France. We observed that weather conditions at sowing differed between locations: (average daily temperatures 3°C colder in Algeria vs. France). For the post-flowering period (PFP), sunflowers in Algeria needed fewer cumulative degree days, in spite of superior thermal requirement for flowering. Cumulative growing degree days required for R6-R9 period was diminished (- 86 °Cd) in Algeria vs. France.

The cumulative growing degree days of vegetative period was negatively correlated with post-flowering period in France with a Pearson correlation coefficient of -0,739. On the contrary there was no significant correlation in Algeria location between VP and PFP.

Effect of early sowing on phenostages

In France, early sowing was one month before control, and crop was harvested with only one week in advance as shown in Fig. 2B. Total cycle duration was longer for 22% on early sowing comparatively to control sowing (159 days vs. 130 days for control sowing). In Algeria, total cycle duration of early sowing was only 8% longer than control sowing. It was sown 3 weeks before the control and harvested 13 days before the control. Proportion of VP in total cycle was mildly longer than in control. In both sites sowing to emergence period increased (multiplied by four in France and by two in Algeria), and emergence to flowering period decreased in response to early sowing.

Thermal time requirement for sunflower development in early sowing compared to control sowing was different between French and Algerian locations, except for flowering duration (from R5 to R6) (Fig. 2C). In France, we observed an increase in the thermal time requirement for the total cycle in response to early sowing. Vegetative stage and PFP present a significant correlation between control and early sowing of -0.336**. On the contrary, in Algeria, early sowing necessitated less cumulative degree days for total cycle (186 °Cd less than control) despite increasing the thermal time requirement for emergence (Fig. 2C).

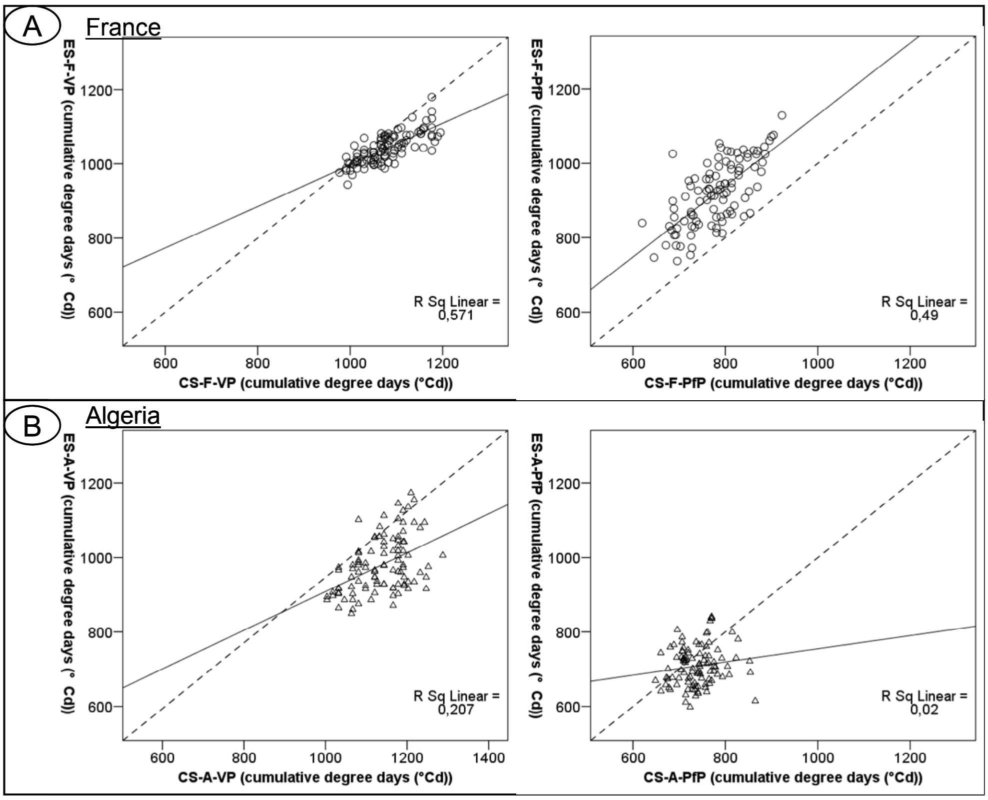

Fig 3. Correlation between control (CS) and early sowing (ES) for vegetative period (VP, left graphs) and post-flowering period (PFP, right graphs) in cumulative growing degree days: in France (A) and in Algeria (B).

Genotypic variation among the 100 recombinant inbred lines of sunflower

Differences among RILs were significant for vegetative period (VP) in all conditions (planting date and site). For example, VP in control sowing ranged from 977 °Cd to 1195 °Cd depending on genotypes in France, and similar amplitude was observed for early sowing. However, genetic differences were less pronounced for post-flowering period in Algeria (PFP ranges from 598 °Cd to 840 °Cd).

High significant correlations were observed between planting dates for vegetative period in both sites (Pearson correlation coefficient was equal to 0.571** and 0.207 in France and Algeria, respectively, Fig. 3). However, non significant correlation was observed between planting date for the post-flowering period in Algeria.

DISCUSSION

Thermal time requirements for sunflower phenostages differed between control and early planting dates. We have shown in Fig. 2C that early sowing in France required more cumulative growing degree days than control sowing, whereas in Algeria early sowing required fewer cumulative growing degree days than control sowing. Cumulative growing degree days were calculated assuming the same base temperature in control and early treatments. Different base temperatures were used in the literature. The base temperature used by Hammer et al. (1982) for VE to R1 stage was 6.6°C whereas Villalobos and Ritchie (1992) and Aiken (2005) used a base temperature of 4 °C. Casadebaig et al. (2008) used a value of 4.8°C as in this study. The value of 4.8°C we have used was probably not suitable for forecasting sunflower phenostages under early sowing conditions.

Differences in the thermal time requirement for sunflower phenostages observed between sites and between early and control sowings could also be explained by photoperiod effect. Aiken (2005) has shown that field observations support earlier reports of long-day photoperiod response for sunflower development to the bud-visible (R1) phenostage; a short day response for development to maturity (R9) was most closely correlated with daylight at the floral initiation. Goyne and Schneiter (1987) have shown that photoperiods of 11 through 13h severely delay the rate of development for most genotypes. Therefore, Goyne et al. (1989) have shown no influence of a photoperiod within the range of 14.5h to 16.2h. In our experiments, daylength differences between early and control sowing and between locations were observed (data not shown). Further investigations on temperature x photoperiod interactions have to be conducted.

A significant variability between genotypes was observed for vegetative period and reproductive period (Fig. 3). Concerning the effect of early sowing on phenostage, we have shown in Fig. 3 that genotype ranking was not affected by early sowing for vegetative stage on the two sites. However, genotype ranking was not preserved for the control sowing during post-flowering stage in Algeria. During the grain filling period (R6-R9) high temperatures were observed in Algeria. Moreover, meteorological data showed that there was no rainfall in Algeria during this period. Drought occurring during this period considerably reduced the variability of the phenostage (R6-R9) and modified the genotype ranking.

Genetic basis of sunflower phenostages response to early sowing must be explored, in terms of genetic variability for base temperature and temperature x photoperiod interactions.

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SUNFLO: A joint phenotyping and modelling approach to analyse and predict the differences in yield potential of sunflower genotypes

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ABSTRACT

This work was focussed on improving the description of organogenesis, morphogenesis and metabolism in a biophysical plant model. A greenhouse experiment was carried out to identify and to estimate the phenotypic traits involved in plant productivity variability of 26 genotypes. The ability of the biophysical model to discriminate the genotypes was tested on previous results of a field survey focussed on evaluating their genetic progress since 1960. Plants were phenotyped on 4 areas: phenology, architecture, photosynthesis and biomass allocation. 12 traits or genotypic parameters were finally chosen to account for the phenotypic variability. A biophysical model was especially built to integrate the genotypic parameters and to evaluate their respective contribution to the variability of yield potential. A large phenotypic variability was found for each term of the energetic approach of above-ground biomass production. The biophysical model was able to account for 80 to 90% of observed variability in yield potential. This model was an interesting tool for analyzing the phenotypic variability of complex plant characteristics such as light interception efficiency. This model showed that several ways are possible to reach high yields. Unlike a classical statistic analysis, this approach allowed to highlight some efficient parameter combinations used by the most productive genotypes. The next steps will be to evaluate the genetic determinisms of the genotypic parameters and to test the reliability of the phenotyping approach.

Key words: biophysical model – *Helianthus annuus* – phenotypic expression – phenotypic characterization – sunflower – yield potential.

INTRODUCTION

Current knowledge in biology does not presently allow to link “whole plant” and “molecular” approaches. As a result, complex plant characteristics such as crop yield cannot be grasped by using “molecular” knowledge in "bottom up" approaches. Consequently, journals are crammed with attempts to identify genes that might explain the build up of plant phenotypes in responses to environment. However, strong difficulties have been encountered in attempting to quantitatively relate the information at gene level to its expression in complex phenotypic traits at plant level (e.g. Sinclair et al., 2004). This actual gap between the identification of an allelic combination at genome level and the corresponding phenotype at plant level greatly limits the potential benefits of the bottom-up approaches in improving our understanding of the genotype-environment interactions and the phenotypic plasticity (e.g. Sinclair and Purcell, 2005). At the same time, the biophysical approaches progressed in understanding and in formalizing the interactions between physical environment and plant responses and regulations (e.g. Jones, 1992).

A possible approach to reducing the gap between the molecular and plant levels is the use of models representing the plant as a biophysical system decomposed as a set of functions determining the phenotype built up in response to environment (e.g. Jeuffroy et al., 2006). To get a coherent system, two types of equations have to be combined, the energy and mass balance equations and the biological regulation equations in response to environment. From one degree of breaking down the plant functioning in elementary processes, the parameters of the equations used to describe these elementary processes may be compared to genotypic characteristics (Yin et al., 2004). Then, it is possible to use the quantitative genetic methods, especially heritability calculations and QTL determinations, to evaluate the genetic determinism and the variability of the studied process. Depending on the way the plant response is taken into account, the use of this genetic information in a set of equations describing the plant functions may allow to account for the plant phenotypic plasticity. This approach has been explored for complex traits such as the expansion rate of a single leaf (Reymond et al., 2003). These examples are far from crop yield, in terms of complexity and time and space levels. The more suitable plant representations to tackle the yield variability would be the crop models (Sinclair and Seligman, 1996). Recent studies have attempted to integrate biochemical and physiological information in crop model to improve the heuristic performance of these models in the analysis of phenotypic plasticity (e.g. Hammer et al., 2004).

The objective of this study was to evaluate the ability of a phenotyping approach combined with a dedicated simple biophysical model to account for the genotypic variability of yield potential. Our assumptions was that the genotypic variability of seed yield could be accounted for by using a set of robust equations, well-tested in crop modelling studies, coupled with a few parameters taking into account the observed phenotypic variability of the studied genotypes. This approach included three parts (i) the development of a biophysical model taking into account the specificities of the sunflower biology, (ii) the estimation of genotypic parameters from measurements on a limited number of isolated plants grown in greenhouse and (iii) an independent set of data obtained in a field experiment for the model evaluation. A panel of 26 genotypes was studied mixing historical commercial hybrids (Vear et al., 2006), experimental hybrids and introgression lines between *Helianthus annuus* and *Helianthus mollis*. This panel was interesting for two reasons. First, the 26 genotypes displayed a wide range of phenotypic differences. Previous observations reported differences related to phenology, light interception, biomass production and allocation (Debaeke et al., 2004). Secondly, there were very large differences in seed yield between genotypes. The seed yield of the most productive genotype is five time higher than the introgession lines. Even among the commercial hybrids, differences higher than 40% were observed (Vear et al., 2003). This trait variability and seed yield scale are relevant for evaluating a modelling approach. The genotypic parameters were chosen according to their ability to integrate the specificities of the sunflower biology in the terms of the Monteith generic approach of the biomass production (Monteith, 1977). Beyond the objective to model the seed yield phenotypic variability from genotypic characteristics, this second aim was to evaluate which plant traits highly contribute to the seed yield variability.

MATERIALS AND METHODS

Model development: The model SUNFLO estimates the above-ground biomass production of a sunflower crop from incident radiation and mean air temperature. It works in daily time steps and describes the plant phenology, the plant leaf expansion, the biomass production and allocation. It takes into account the behaviour of various genotypes by the mode of some parameters which are genotype dependent.

The plant phenology is driven by the thermal time. Cumulative thermal time was calculated as the sum of the daily mean air temperature from emergence using a base temperature of 4.8°C common to all genotypes. Four key stages, expressed as thermal dates with genotypic values, were used to delimited periods of plant cycle with changes in plant physiology: the floral bud appearance (E1), the beginning of flowering (F1), the beginning of grain filling (M0) and the physiological maturity (M3) (Table 1).

Assuming the canopy is a homogeneous absorber, the daily radiation interception efficiency (RIE) was estimated from Beer's law using daily LAI and an extinction coefficient (k) determined for each genotype (Table 1). LAI was calculated from the plant density and the plant leaf area able to intercept photosynthetically active radiation. This latter was estimated as the difference between total leaf area and senescent leaf area. Because in sunflower the distribution of the leaf area along the stem showed a bell-shape (Dosio et al., 2003), plant leaf area was calculated from leaf number (N) with a logistic equation with 3 genotypic parameters, A1, A2 and A3, respectively, the maximal plant leaf area, the rank and the area of the largest leaf of the plant (Table 1). The number of leaves increases linearly with cumulative thermal time from emergence to the beginning of flowering. Then the leaf number decreases linearly from the beginning of seed filling to plant maturity as nitrogen moves from leaves to seeds during the monocarpic leaf senescence (Sinclair and deWit, 1975).

The radiation use efficiency (RUE) represents the ability of the crop to convert the intercepted energy into biomass. RUE is known to change during the plant growth cycle (e.g. Lecoeur and Ney, 2003 on pea). A single general pattern of change in RUE over crop development was used for all genotypes. RUE was equal to a minimum up to 300°Cd, then it increased linearly to reach a maximum level at the beginning of flowering. RUE remained constant until the beginning of seed filling, then it declined exponentially to zero upon the plant death. This general pattern was modulated through a genotypic parameter taking into account the different photosynthetic capacities of genotypes relative to Melody. A depressive function of non optimal temperature was applied to RUE, calculated from daily mean air temperature. The above-ground biomass production was calculated from Monteith's formula (1977) linking dry matter production to incoming photosynthetically active radiation through two radiation efficiencies.

The allocation of biomass to seeds was estimated by using two allocation coefficients. The first coefficient determined the fraction of total biomass allocated to the capitulum (HIcap). It changes with thermal time and was modelled as a single logistic function. However, its maximum value reached at physiological maturity was genotype-dependent. A second coefficient corresponded to the fraction of capitulum biomass allocated to the seeds (HIseed). It was also genotype-dependent. Finally, the seed yield was calculated by multiplying the final biomass and the two allocation coefficients.

Estimation of the genotypic parameter: The estimation of the genotypic parameters was carried out in a greenhouse experiment (Montpellier, southern France). Plants were grown in pots of 7.5 l filled with mixtures of loamy soil and organic compost. There were 10 pots per genotype and they were arranged in order to mimic an agronomic culture density of 6 plants per m². The soil was continuously maintained at water retention capacity by irrigations at least once a day with a modified one-tenth Hoagland solution corrected with minor nutrients. Air temperature and radiative conditions were managed in order to obtain thermal conditions and photoperiod similar to those classically observed in field conditions.

The dates of occurrence of developmental stages E1, F1, M0 and M3 were determined according to the notation proposed by the CETIOM for sunflower on six plants per genotype, twice a week. In addition, the number of visible and senesced leaves were also counted. Architectural measurements were made at the end of flowering when all the vegetative organs were fully expanded. Height, length, width and distance from stem of each blade were measured on 6 plants per genotype with a ruler (± 0.5 mm). In addition, their zenithal angles were measured with a digital protractor (± 0.5°, Pro 360, Mitutoyo, Paris, France). Then, the individual leaf area was estimated from an allometric relationship with the length and the width of the leaf blade. The light interception efficiency, and, thus, the extinction light coefficient (k), were estimated using 3D virtual scenes and a radiative balance model (Rey et al., 2008). The photosynthetic parameter (PS) was estimated from leaf photosynthetic activities measured with a portable photosynthesis system (CIRAS, PP system, UK) with a control radiation level of 1500 µmol m^{-2} s^{-1}. All the genotypic values were normalized with respect to those obtained for Melody.

Yield index and model validation: For a given genotype, the yield index (I) was defined as the ratio between the seed yield of this genotype and the average seed yield of the five oldest genotypes (see Vear et al., 2003 for more details). A yield index was calculated (Imod) for each of the 26 genotypes running SUNFLO with the mean climate conditions observed in Montpellier during the last 25 years. Imod was compared to a reference yield index (Iref). For the genotypes 1 to 20, Iref was taken from Vear et al. (2003). Iref of genotypes 21 to 26 came from experiments where some of genotypes 1 to 20 were grown in addition to the considered genotypes (Vear, pers. comm. for genotypes 21 to 23; Seryes, pers. comm. for genotypes 24 to 26).

Model simulations were tested with an independent data set collected in a field experiment carried out in Montpellier in 2002 with 5 genotypes (Albena, Heliasol, Melody, Prodisol and Vidoc) (see Rey et al., 2008 for more details on experimental design and measurements).

RESULTS

12 phenotypic traits displayed statistical differences between the 26 genotypes and were then considered as genotypic parameters (Table 1). The architectural parameters presented the highest variability with a CV value higher than 20%. The thermal date of the four key developmental stages presented a similar range of variation with CV around 10%. More surprising was the high variability in parameter k, which corresponds to the efficiency of the plant leaf area to intercept the incident radiation. This parameter is generally considered as a species characteristic and close to 0.80 for cultivated sunflower. The obtained range of k values is close to what is observed in the plant kingdom. The 10% difference in photosynthetic activity (PS) was also surprising because it was observed to be among the best commercial genotypes which are considered as optimized on this trait. In term of biomass allocation, almost no variability was observed in the proportion of biomass allocated to the capitulum (HIcap). On the other hand, the capitulum biomass allocated to the seed (HIseed) displayed a high variability with a significant increase in this value among the recent commercial genotypes.

Table 1. Minimum, maximum and mean values of the 12 genotypic parameters displaying significant differences between the studied genotypes

Genotypic parameter		Minimum	Maximum	Mean	CV
E1	(CDD)	425	690	525	0.12
F1	(CDD)	863	1253	989	0.09
M0	(CDD)	1136	1460	1253	0.07
M3	(CDD)	1578	2242	1772	0.09
Nmax	(#)	18.8	42.5	27.3	0.20
A1	(cm²)	1939	7430	5095	0.25
A2	(#)	11.0	31.5	14.9	0.25
A3	(cm²)	138	466	343	0.21
K		0.52	0.96	0.85	0.11
PS		0.92	1.02	0.97	0.03
Hicap		0.5	0.55	0.52	0.03
Hiseed		0.40	0.70	0.59	0.14

The ability of SUNFLO to account for the crop functioning was evaluated by comparing the values of a set of simulated variables to observed independent values obtained in Montpellier in 2002. The chosen variables tested the model performance on its major parts which are phenology, architecture and biomass production and allocation (Table 2). A good consistency was seen between observed and simulated values whatever the considered variables. The mean errors on phenology, architecture and total biomass were close to 10% of the observed values. The capitulum biomass displayed the highest mean error with approximately 30%.

Table 2. Comparison of observed or simulated values of the SUNFLO model.

Variables	n	slope	R²	Mean	RMSE	Bias
Number of leaves	62	1.118	0.949	16.18	2.14	-0.0214
RIE	90	1.011	0.744	0.83	0.088	0.003
Total biomass (g)	35	0.962	0.970	752.1	85.4	14.99
Capitulum biomass (g)	32	0.978	0.904	263.5	77.1	28.32

The yield was simulated for the 26 genotypes with the mean climate data observed in Montpellier during the last 25 years. The emergence date was set on the 15th April. The simulated yield ranged from 119 to 716 g m^{-2} of seeds with a mean value of 447 ± 48 g m^{-2}. The highest simulated yields were close to the values considered as being the biological potential of present genotypes (Connor and Hall, 1997). The simulated yield indices of commercial hybrids ranged from 85 to 167 for, respectively, Peredovik and Melody. The introgression lines had much lower yield indices of below 60. The comparison between the observed and simulated yield indices showed a good consistency between both values (Fig. 1). The slope of the linear regression between observed and simulated values is equal to 1 and the model accounted for more than 80% of the observed variability in the yield index. The mean quadratic error indicated that the model is able to distinguish, in terms of productivity, groups of 3 to 4 genotypes with close yield indices.

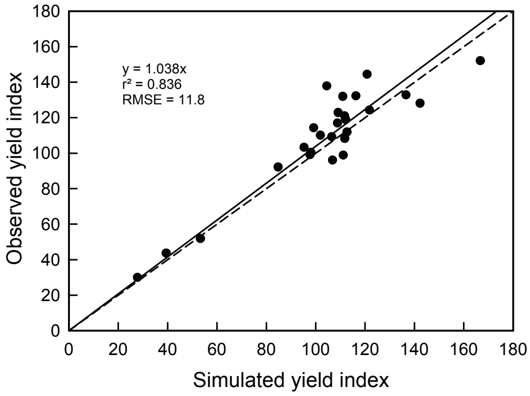

Fig. 1. Comparison of observed and simulated yield indices.

The impact of the variability of the genotypic parameters on the potential yield was estimated through a coefficient of variation (Fig. 2). To estimate this coefficient of variation, a mean value was imposed for all the genotypic parameters except one. The yield indices were then estimated by using the values observed for the 26 genotypes. This approach predicts the existing variability in *Helianthus annuus* species. As the parameter impacts are not strictly additive, the sum of the individual impacts was higher than the total observed variation in yield indices. However, this approach gave some information on the relative weight of the genotypic parameters in the yield variations.

The impact of the genotypic parameter values on the coefficient of variation of seed yield ranged from 0.5% to 14.3% for, respectively, the thermal date of E1 and the coefficient of capitulum biomass allocation to the seeds (HIseed). The other strongest individual impacts were observed for the thermal dates of F1, M0 and M3 and for the maximum plant leaf area (A1) and the position of the largest leaf (A2). When the parameters were bulked according to the major parts of the model, the ranking of the processes in term of their impact on yield variability was, first, the biomass allocation and the light interception through the plant architecture, second, the plant phenology and, far away, the photosynthesis.

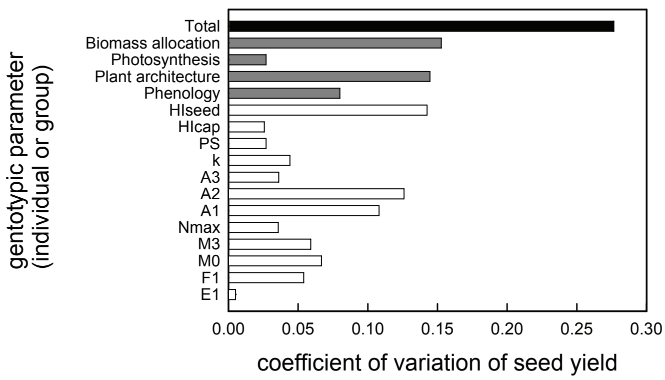

Fig. 2. Relative impact of individual or group of genotypic parameters on simulated seed yield.

DISCUSSION

The proposed approach combining phenotyping and modelling appeared to be relevant for analysing complex phenotypic traits such as seed yield. The estimation of the genotypic parameters on a few plants grown in a greenhouse gave values close to those usually observed in field conditions (Vear et al., 2003). This approach also revealed interesting traits rarely taken into account such as plant architecture, light interception efficiency and photosynthesis. The next steps would be to evaluate the robustness and the reliability of such phenotyping approaches. The more relevant traits might be the target of a more detailed analysis, especially in terms of their genetic determinism (Triboi et al., 2004). At present, this analysis would be greatly slowed down by some phenotypic measurements which are time-consuming. This suggests that simple and rapid methods in measuring phenotypic traits have to be developed. With a broader and genetically organized panel of genotypes, statistical analysis of the parameter combinations or associations may allow to identify different ideotypes. It may also allow a subsequent analysis of the breeding strategies.

The simple biophysical model SUNFLO was able to account for approximately 80% of the variability in potential seed yields. This result was obtained with a highly contrasting panel of genotypes in terms of productivity. This is promising, although the resolving power of the model is still insufficient. A ten point uncertainty in yield indices is still too high to distinguish the genotypes of one same breeding generation. However, the modularity of the biophysical model is of interest for identifying the strong and weak points of a given genotype. For instance, some genotypes were very efficient in biomass allocation or in photosynthesis but none of them were optimized for all the processes described in the model. This suggests that some progress margins still exist in terms of productivity. These margins may be defined by looking for original combinations of the genotypic parameters corresponding to unknown virtual genotypes, which could be tested *in silico*. This last approach might be a useful tool for increasing the efficiency of breeding programs.

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Root system and water extraction variability for sunflower hybrids

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ABSTRACT

Root traits and soil water extraction of fifteen genotypes were characterized in five greenhouse experiments. The objective was to evaluate the genotypic variability and to identify possible new strategies in plant breeding for drought-stressed conditions. The root traits were characterized at the flowering stage by the root length density (RLD) and the effective rooting depth (Z). The performance in soil water extraction was characterized by the fraction of extracted soil water (EW). It was estimated from soil drying experiments conducted on plants at different stages. Z and EW were used to calculate an indicator of the amount of extractable soil water (EW_{gen}). Wide variability of those traits was observed among the genotypes. Four classes of genotypes were found with a maximal difference of 10% between the extreme values of fraction of extracted soil water. Water depletion kinetics was different between the experiments but the fraction of extracted soil water was stable for each genotype. A large genotypic variability for the indicator of the extractable soil water was also observed. This variability resulted from different combinations of effective rooting depths and fractions of extracted soil water. These traits might be of interest for breeding cultivars well adapted to water stress conditions.

Key words: drought stress – extracted soil water – genotype – rooting depth – root length density – sunflower.

INTRODUCTION

Water deficit is the most predominant abiotic stress experienced by sunflower (*Helianthus annuus* L.) especially because most sunflower crops are cultivated under rainfed conditions (Goyne et al., 1978; Yegappan et al., 1982; Connor et al., 1985). To sustain production in such limiting environmental conditions, sunflower drought tolerance should be increased. It could be done through the selection of plants able to limit the water deficit they undergo under limited soil moisture conditions. One way could be to improve the plant performance in soil water extraction, either by increasing the soil depth explored by roots (Connor and Hall, 1997) or by increasing the fraction of soil water extracted by the plant.

The objective of this study was to evaluate the genotypic variability in the root system architecture and in the soil water extraction for a panel of commercial genotypes. Five greenhouse experiments were conducted between 2005 and 2007 on 15 genotypes. They represented 40 years of currently used cultivars; 10 are old and modern hybrids currently cultivated in France and 5 are experimental hybrids, which could be the next cultivars in France (F. Vear, pers. comm.). The root traits were characterized by the root length density and the effective rooting depth. The performance in soil water extraction was characterized by the fraction of extracted soil water. It was estimated at the end of a drying cycle.

MATERIALS AND METHODS

Plant materials and growth conditions

Five experiments were conducted in a greenhouse in Montpellier (France, 43°35'N and 3°58'E) from 2005 to 2007 (Table 1). 15 genotypes with contrasted phenology, architecture, photosynthesis and productivity were studied (Table 2). Plants were grown in plastic pots in Exp. 1 to 4 and in PVC columns in Exp. 5 filled with a mixture of loamy soil, sand and compost at the same volume. Each genotype was characterized by 6 plants in Exp. 3, 4, 5 and five plants in Exp. 1 and 2. Pots were installed in order to obtain a culture density of six plants per square meter. In order to avoid water deficit, plants were irrigated four times per day with a one-tenth Hoagland solution corrected with minor nutrients. Irrigation was stopped when the plant had 6, 12 or 14 full-expanded leaves respectively in Exp. 1, 3 and 4. In Exp. 2 irrigation was stopped when the plant reached the floral bud stage E1 (CETIOM, 2004). The natural light in the greenhouse was supplemented with sodium lamp (250 $\mu mol\ m^{-2}\ s^{-1}$) giving a photoperiod of 12h. Temperature in the greenhouse was maintained between 16°C and 30°C. Environmental conditions for the experiments are summarized in Table 1.

Table 1. Mean characteristics of the five experiments

Exp N°	Sowing date	Mean value of Temperature (°C)	Mean value of Vapour Pressure deficit (kPa)	Mean of daily cumulative PPFD (mol $m^{-2}d^{-1}$)	Number of genotypes
1	21 November 2005	18.4	2.12	25.13	15
2	19 November 2006	23.1	2.84	26.19	10
3,5	15 February 2007	23.5	2.91	23.58	10
4	3 April 2007	23.3	2.87	32.69	10

Table 2. Studied genotypes in the different experiments and their registration years

Genotype	Exp N°	Registration year
Peredovik	2, 3, 4	1960
Primasol	1, 2, 3, 4	1978
Albena	1, 2, 3, 4	1988
Vidoc	1, 2, 3, 4	1989
Santiago	2, 3, 4	1993
Melody	1, 2, 3, 4	1996
Sanbro	2, 3, 4	1997
Prodisol	1, 2, 3, 4	1998
LG5660	1	1998
Pegasol	1	2001
VAQxPAR6	2, 3, 4	2003[1]
VDQxOPB4	1	2003[1]
VDQxPPR9	1	2003[1]
XRQxPPR9	1	2003[1]
XRQxPST5	2, 3, 4	2003[1]

[1]Experimental breeding year

Measurements

Environmental conditions were measured continuously for all experiments. Air temperature and relative humidity were measured with a capacitive hygrometer (HMP35A Vaisala, Oy, Helsinki, Finland). Incident photosynthetic photon flux density (PPFD) was measured with a quantum sensor (Campbell PKS 215, Campbell Scientific Ltd, Shepshed, Leicestershire, England). Data were collected every ten seconds and means were stored every 1800s in a datalogger (CR10, Campbell Scientific Ltd).

Plant leaf area was estimated just before stopping irrigation in Exp 1 to 4 and at flowering stage in Exp. 5, by measuring the length and width of leaves. In Exp. 5, soil column was stratified per 10 cm for the first 20 cm layer and per 20 cm for the next. In each layer, roots were harvested and separated in thin or "structural" roots. Roots with a diameter of less than 2 mm were considered as thin. A 2-meter thin roots sample was picked from the first 10 cm soil layer. The root dry weight of this sample (DW_{2m}) and the DW (g) of the two classes of roots were estimated after drying at 60°C for 48h. The root mass length (Lm, cm g^{-1}) was calculated as the thin root length per unit of thin root mass:

$$Lm = 200 / DW_{2m} \qquad \text{(Eq. 1)}$$

The root length density (RLD, cm cm^{-3}) is the length of thin roots per unit of soil volume explored by the root system. It was calculated for each soil layer as follows:

$$RLD = \frac{Lm \; DW_{thin}}{V} \qquad \text{(Eq. 2)},$$

RLD, root length density (cm cm^{-3}); DW_{thin}, dry weight of thin root in the considered soil layer; V, volume of the considered soil layer.

The effective rooting depth for water extraction (Z, cm) was estimated as the root depth for which the root length density was more than 1cm cm^{-3}. As proposed by Gregory (1994), Z was determined from linear regression between the depth of a layer (Y, cm) and the logarithmic value of the root length density.

$$Y = a \ln RLD + Z \qquad \text{(Eq. 3),}$$

Y, soil depth; a, coefficient of root length density distribution; RLD, root length density; Z, effective rooting depth.

In Exp. 1 to 4, a drought stressed treatment was applied stopping the irrigation at a determined phenological stage. The evening prior to the beginning of the treatment, all pots were fully watered and allowed to drain overnight. The following morning, pots were weighed to determine the initial soil water content (SWC_i). To prevent soil evaporation, the pots were enclosed in plastic bags. The plant transpiration rates were estimated by weighing each pot every day. The lower limit of soil water content (SWC_{min}) was assumed to have occurred when the plant transpiration remained constant during several successive days and reached 10% or less than that of well watered plants.

The soil water content (SWC, g g^{-1}) was estimated by weighing soil samples after drying at 105°C during 72 hours.

$$SWC = \frac{FW_{soil} - DW_{soil}}{DW_{soil}} 100 \qquad \text{(Eq. 4)}$$

SWC, soil water content; FW_{soil}, soil fresh weight; DW_{soil},soil dry weight

The fraction of soil water extracted by the plant (EW) was estimated as follows:

$$EW = \frac{SWC_i - SCW_{\min}}{SWC_i} 100 \qquad \text{(Eq. 5)}$$

Estimation of the amount of extractable soil water

The effective rooting depth (Z) and the fraction of soil water extracted by the plant (EW) were used to calculate an indicator of the amount of extractable soil water for each genotype (EW_{gen}, mm) relative to a standard condition. The chosen reference was a sunflower with an effective rooting depth of 1800 mm (Angadi and Entz, 2002) growing in a soil with 0.13 mm mm^{-1} of available soil water (Ratliff et al., 1983). EW_{gen} was calculated as follows:

$$EW_{gen} = \left[\frac{EW_i}{\frac{1}{n}\sum_{i=1}^{n} EW_i} \; 0.13 \right] \left[\frac{Z}{\frac{1}{n}\sum_{i=1}^{n} Z_i} \; 1800 \right]$$

(Eq. 6)

EW_{gen}, amount of extractable water for the genotype i; n, number of studied genotypes (10)

RESULTS AND DISCUSSION

Root traits: root length density and effective rooting depth

As illustrated in Fig. 1, the pattern of the evolution of the vertical distribution of root length density was similar for all the genotypes. The root length density decreased exponentially with soil depth. 85% of root length density was observed in the first 40 cm of soil depth (Fig. 1). These results obtained in pot experiments are consistent with previous works in field experiments (Sadras et al., 1989; Cabelguenne et al., 1999; Angadi and Entz, 2002) showing a conical root system. Nevertheless, a large genotypic variability in root length density was observed, especially in the first 0.40 m depth. The mean root length density in the top 1 m soil depth was significantly different between genotypes (Fig. 1 and Table 3). This value varied from 2.39 (Primasol) to 7.65 cm cm^{-3} (XRQ x PST5). Similar genotypic differences in root distribution were reported by Angadi and Entz (2002).

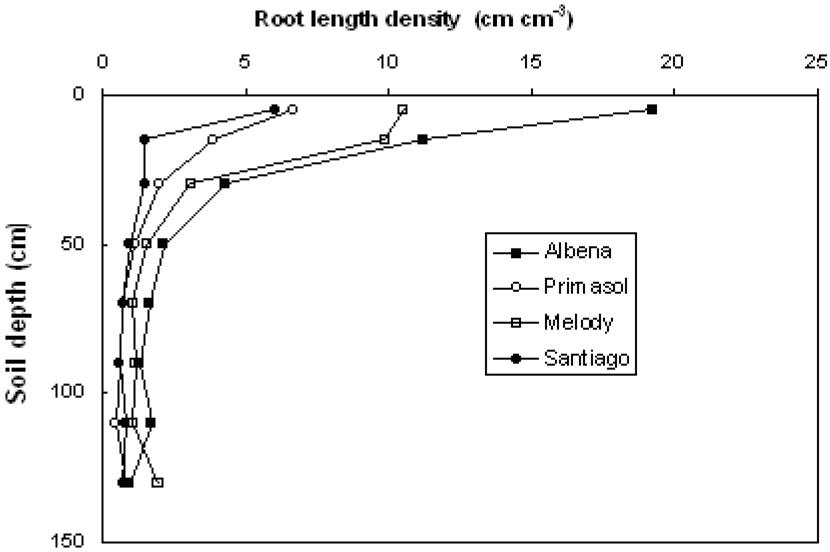

Fig. 1. Vertical distribution of root length density of four contrasted genotypes. Each point is the mean of 6 plants.

Table 3. Effective rooting depth and root length density. Values are average of 6 plants. Genotypes with the same letters did not differ significantly (α = 5%)

Genotype	Effective rooting depth (cm)	Mean root length density in the top 1 m soil depth ($cm\ cm^{-3}$)
Peredovik	87 abc	5.10 abcd
Primasol	68 b	2.39 a
Albena	105 a	5.69 bcd
Vidoc	81 abc	6.17 cd
Santiago	71 bc	2.45 a
Melody	94 ac	4.52 abc
Prodisol	82 abc	2.91 ab
Sanbro	99 a	4.34 abc
VAQxPAR6	99 a	5.35 abcd
XRQxPST5	104 a	7.65 d

A large genotypic variability was observed for the effective rooting depth (Table 3). Values ranged from 68 cm (Primasol) to 105 cm (Albena). Three classes of genotypes were found, one with an effective rooting depth of below 71 cm, one with an effective rooting depth of over 99 cm and the last one with intermediate values. As all the genotypes were cultivated in identical soil columns, the differences could be attributed to genotypic plant characteristics. Nevertheless, it is worth noting that effective rooting depth in sunflower is also dependent on soil characteristics (Meinke et al., 1993). Different combinations of effective rooting depths and root length density were observed. Some genotypes with an effective rooting depth over 99 cm presented a high RLD as XRQxPST5 or a moderate one as Sanbro (Table 3). Other genotypes with an effective rooting depth of between 71 cm and 99 cm presented a low RLD like Prodisol or a high one such as Vidoc (Table 3).

Fraction of extracted soil water

The comparisons of the soil water depletion kinetics in experiments 1 to 4 revealed significant differences in the mean duration of pot desiccation between cultivars (data not shown). This resulted from differences in environmental conditions between experiments but also from differences in the initial developmental stages of the plants. But variability for soil water depletion duration did not have any influence on the fraction of extracted soil water between the genotypes. The fraction of extracted soil water (EW) showed significant differences between genotypes (Table 4). Five classes of genotypes were found with a maximal difference of more than 10% between the extreme ones. For example, EW varied from 82.7% for the experimental hybrid VDQxOPB4 to 69.8 % for Peredovik.

Table 4. Fraction of extracted soil water. Values are the average for 5 or 6 plants.

Genotype	*Fraction of extracted soil water*[1] (%)	
Peredovik	69.8	a
Primasol	71.3	abc
Albena	75.1	bcd
Vidoc	75.7	bcd
Santiago	71.4	abc
Melody	70.4	ab
Sanbro	75.8	cd
Prodisol	73.9	abcd
LG5660	73.1	abcd
Pegasol	73.9	abcd
VAQxPAR6	74.8	abcd
VDQxOPB4	82.6	e
VDQxPPR9	76.7	d
XRQxPPR9	70.5	ab
XRQxPST5	71.7	abcd

[1]Genotypes with the same letters did not differ significantly ($\alpha = 5\%$)

These classes were globally the same in the four experiments (Exp. 1 to 4). This result shows that the water extraction ability in sunflower was quite stable and it might be under genetic control. The stability and the heritability of EW should be studied in further experiments.

Genotypic extractable soil water

Significant differences in the indicator of the extractable soil water (EW_{gen}) were observed between genotypes (Fig. 2). Values ranged from 169, for Primasol, to 283 mm for Sanbro. This leads to a maximum difference of 114 mm between the genotypes studied corresponding to 28 - 38% of the amount of water used for a sunflower crop in West of Europe, which is about 300 to 400 mm. In this study, EW_{gen} was estimated for a reference soil with 0.13 mm mm^{-1} of available soil water (Ratliff et al., 1983). This range could be wider under field conditions. Indeed, the amount of available water for a crop depends either on plant or soil characteristics. For one cultivar of sunflower, Meinke et al. (1993) have found a total plant available water for the root profile ranging from 77 to 210 mm for a wide range of soil types.

The variability in EW_{gen} resulted from different combinations of effective rooting depths and fractions of extracted soil water. The lower EW_{gen} was observed in Primasol (Fig. 2), which combined a low effective rooting depth (Table 3) and a low fraction of extracted soil water (Table 4). Intermediate values of EW_{gen} were observed for low or high fraction of extracted soil water as for Melody and Prodisol (Fig. 2 and Table 4). Finally, the best performing genotype for water acquisition was Sanbro, which combined a high effective rooting depth (Table 3) and an intermediate fraction of extracted soil water (Table 4). These results are consistent with those of Angadi and Entz (2002) who attributed greater soil water depletion to deeper rooting depth. No genotype presented both a high effective rooting depth and a high fraction of extracted soil water. No correlation was found between EW_{gen} and the registration year of the genotypes (Table 2). That means that an unexplored source of variability could be used by the breeders to improve sunflower productivity.

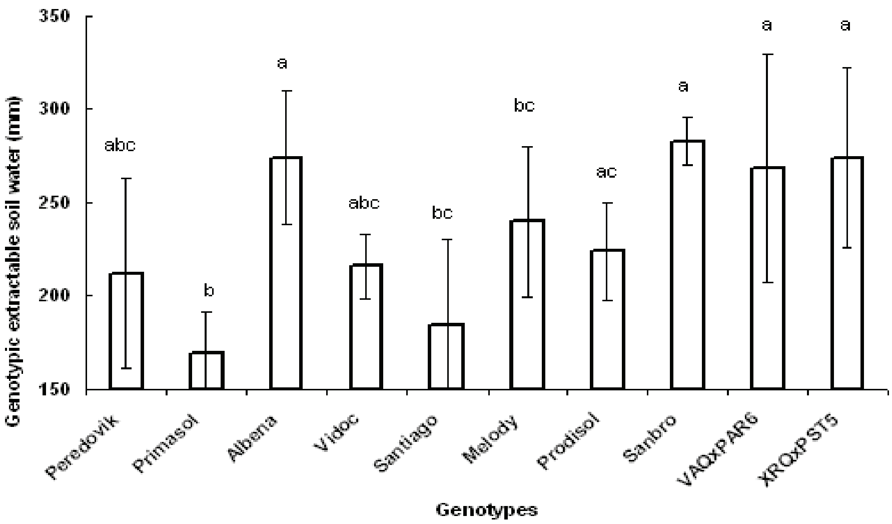

Fig. 2. Amount of extractable water of ten cultivars. Each point is the average of 6 plants. Vertical bars represent the standard deviation. Genotypes with the same letters did not differ significantly (α=5%)

CONCLUSIONS

This study showed a large genotypic variability for the root traits and the soil water extraction: root length density, effective rooting depth and fraction of extracted soil water. No correlation was found between EW_{gen} and the registration year of the genotypes, nor between effective rooting depth and fraction of extracted soil water. The modern genotypes are not better in soil water extraction than old ones. The effective rooting depth and the plant ability to extract soil water could be interesting targets for sunflower breeding programs. Ideotype with a deep root system and a low root density would be suitable under deep soil conditions. In contrast, ideotype with a small deep root system and a high root density would be suitable under shallow soil and limited water conditions.

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Effects of high water table conditions on sunflower growth and quality

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ABSTRACT

Sunflower is one of the major crops for edible oil, which is widely known as a high quality and healthy oil. Recently it was reported that the oil of high oleic acid cultivars had higher oxidation stability and better dietary properties than that of the standard linoleic cultivars. In Japan, sunflower is frequently cultivated as an upland-crop component in rice-based cropping systems. Therefore, an understanding of the effects of water conditions on the growth and oil quality is important. The aims of this paper are to evaluate the effects of water conditions on plant growth, seed yield and oil quality, and to obtain physiological information for increasing yield of sunflower in rice-based upland fields in the central region in Japan. Seed yield and the major yield components, as well as the oil content, were negatively affected by the shallower water table and lower temperature conditions. Regarding their fatty acid composition, the percentage of oleic acid was decreased, and that of linoleic acid was increased with the higher water table. In the next step, the physiological mechanisms behind the effect of higher water table should be elucidated to develop improved management practices for increasing the seed yield and improving the oil quality of sunflower in rice-based cropping systems.

Key words: fatty acid – rotational upland paddy field – sunflower – water table level.

INTRODUCTION

Regarding the effects of short-term waterlogging on sunflower, Orchard and Jessop (1984) reported that the yield was most affected by the waterlogging at anthesis. They also reported that waterlogging at the vegetative and floral initiation stages inhibited the leaf expansion (Orchard and Jessop, 1984). Regarding the soil conditions affected by waterlogging, Orchard and So (1985) reported that availability of oxygen concentration was reduced and ethylene concentration was increased. Hunt et al. (1981) reported that this increased ethylene concentration affected plant growth and reduced root growth in tobacco. Grassini et al. (2007) reported that waterlogging during grain filling caused direct and adverse physiological responses: leaf area, leaf capacity to fix carbon, water absorption, and grain yield were all decreased.

As mentioned above, there are some reports about the effect of waterlogging on sunflower growth, but there are few reports about the effect on oil quality. In the case of soybean, Shimada et al. (1995) reported that the depth of the water table affected chlorophyll contents and yield. The effects on chlorophyll contents varied with the leaf position on the main stem. When the plants were grown with a shallower water table, the lower leaves contained less chlorophyll, and the ripened pod number and 100-seed weight were decreased.

Regarding the fatty acids, there were some reports on the factors changing their compositions. Since waterlogging conditions usually delay the growth stages, the air temperature during the grain filling would be different among the treatments, so that the temperature condition was widely reported as being one of those factors. Nagao and Yamazaki (1984), Sobrino et al. (2003) reported that the oleic/linoleic acid ratio was increased with higher temperature during grain filling. Izquierdo et al. (2006) reported that night minimum temperature during grain filling was better related to oleic acid concentration and its linear increase increased oleic acid concentration. Flagella et al. (2002) reported that oleic acid and stearic acid were decreased and linoleic and palmitic acid were increased under irrigation. But not many papers have been published regarding the change in quality under high water table conditions.

In Japan, the cultivation of sunflower on rice-based upland fields for the purpose of human consumption, and for the use of bio-diesel fuel is increasing. Therefore, the objective of this paper is to elucidate the effects of a shallow water table on the growth, yield and quality of sunflower.

MATERIALS AND METHODS

Field experiments

Experiment I was conducted in 2005 in two farmers' upland fields after irrigated rice at Tsukubamirai city in Ibaraki prefecture. Soil moisture conditions were different between the two fields (Fig. 1). One field (F7) was wetter than the other (E7). Twenty-one hybrid varieties of sunflower were cultivated in them. In 21 cultivars, 9 were linoleic type, 11 were middle oleic type and one was high oleic type. They were sown on May 30 and 31 at 30 x 60cm or 30 x 80cm spacing. A compound fertilizer was applied at the rate of $N\text{-}P_2O_5\text{-}K_2O$ = 8.4-8.4-8.4 g/m^2.

Stem length, disk diameter, number of seeds in a disk, flowering date, and maturing date were measured. Soil moisture content was measured by a soil moisture probe (Profile Probe PR2, Daiki Rika Kogyo Co. Ltd., Tokyo) at depths of 10, 20, and 30cm. Yield of each plot was determined from harvest of 1.44m2 . All treatments were replicated two or three times depending on the varieties.

Experiment II was conducted in 2007 on an artificially sloped plot at Ibaraki Agriculture Institute at Ryugasaki city in Ibaraki prefecture. The slope, 8.3 m in length and 86 cm in height at one end gave a set of 10 rows with different water-tables. The ditch surrounding the sloped plot was constantly filled with water after June 7. Two hybrid varieties were used. One was traditional type; Hybridsunflower (Kaneko Seeds Ltd. Gunma), and the other was NuSun type, 63M80 (Pioneer Hi-Bred International, Inc., USA). These varieties were sown on June 7 and June 26, 2007. The sowing space was at 20 x 86cm. The ditches around the sloping plot were filled with water from June 7 until the end of the experiment. Soil moisture contents were measured as in Exp. I at a depth of 20, 40, 60 and 80 cm. Fertilization and observed parameters were the same as in Exp. I.

Sample and data analysis

Sampled seeds were air-dried. Two g seeds from each sample were crushed and the oil was extracted with *n*-butyl alcohol. The measurement of oil content and fatty acids composition in total fatty acid were determined by the Caviezel method (Pendl et al., 1998) using a gas chromatograph (B-820, Nihon Büch Co. Ltd., Tokyo). The content of fatty acids was calculated from the peak areas and calculated as the percentage of the total fatty acid content.

Statistical analysis

The results were analyzed by ANOVA. All statistical analyses were performed with SPSS 11.0 for Windows (SPSS, 2001). All values are expressed as mean values. Significant statistical differences between treatments were established by the Tukey's test at $P<0.05$. A correlation was calculated with the values of the different parameters. The significance levels ($P<0.01$) are based on the Pearson coefficients.

RESULTS

Exp. I.

Soil moisture conditions in the sloping plot

The first flowering date was July19. and the last maturing date was Sept. 13. During this period, soil water content was always higher in F7 than in E7. The largest differences were observed in the row with the water-table of 20cm in depth (Fig. 1).

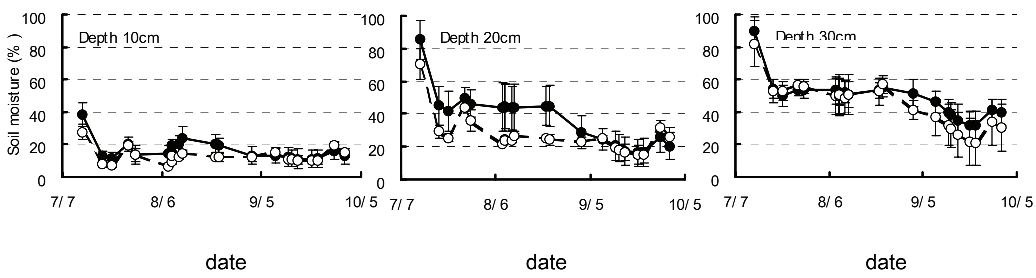

Fig. 1. Soil moisture conditions in the two fields. F7 ●, E7 ○

Effects of soil moisture conditions on growth, yield and quality

The differences in growth and yield between the two fields are shown in Fig. 2. The reductions in stem length in F7 (higher soil moisture condition) were measured in all cultivars. The responses of numbers of seeds in a disk were different depending on the cultivars: that of Hybridsunflower increased and that of 63M02was decreased in F7 field. Their yields in F7 field (higher soil moisture condition) were almost always lower than those in E7 (lower soil moisture condition) (Fig. 2). In this study, the decrease in oil content and oleic acid composition and the increase in linoleic acid composition were observed at the harvest in higher soil moisture conditions. But the order of high or low oil content and oleic acid and linoleic acid composition in cultivars was unchanged in two water table conditions. Regarding the correlations of some traits with the yields, numbers of seeds in a disk had a significant correlation with the yields in both fields (data not shown).

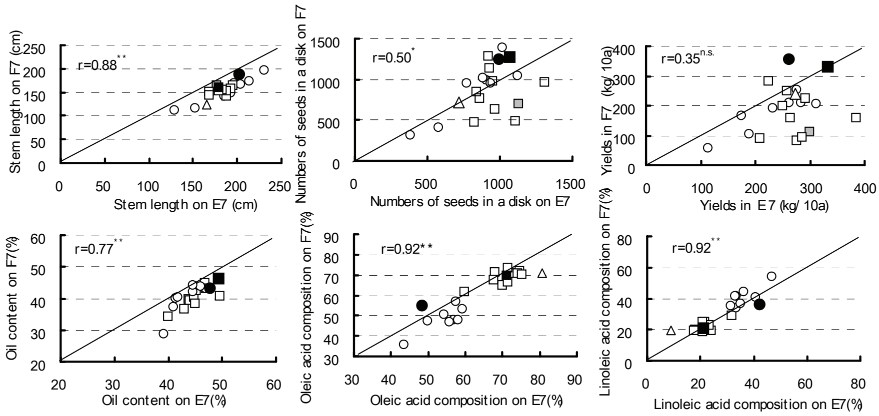

Fig. 2. Growth, yield and harvest quality in the two fields.
○:Trad. (●:Hybridsunflower),□:NuSun (■:63M80, ▩63M02),△:High oleic

Exp. II.

Effects of soil moisture conditions on growth, yield and quality.

Soil moisture contents at different depths in the sloping plot are shown in Fig. 3. The largest difference in soil moisture content was found at the depth of water table of 40 cm. The mean air temperature during the ripening period of each treatment (water-table depth) is shown in Fig. 4. Except for the cases at depths of 0 cm and 9.2 cm, there were no large differences in mean temperature and accumulated temperature (data not shown) between the treatments of the same sowing date (Fig. 4).

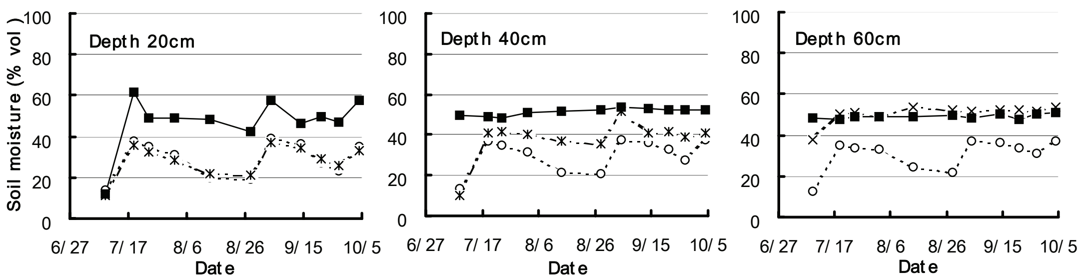

Fig. 3. Soil moisture conditions on the sloping field. Depth to water table; ○:86cm, ×:47.6cm, ■:9.2cm

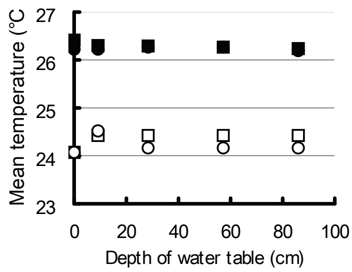

Fig. 4. Mean temperature during ripening period.
Sown on June 7.●Hybrid. (Trad.) ■63M80 (NuSun)
Sown on June 26.○Hybrid. (Trad.) □ 63M80 (NuSun)

The effect of different water table conditions on the growth of sunflower is shown in Fig. 5. Stem elongation, diameter of a disk and yields were significantly reduced with shallower water table. The stronger response to the water table rise was seen when the depth of water table was shallower than about 30 cm (Fig. 5). The growth stage when the water treatment started was different between the plants sown on June 7 and those sown on June 26. The decrease in oleic acid composition ratio and the increase in linoleic acid composition ratio with a rising water table were somewhat clearer for the plants sown on June 7 (Fig. 5).

These experiments demonstrated that the shallow water table affected not only the growth and yield but also the quality of the harvest even under the same temperature conditions.

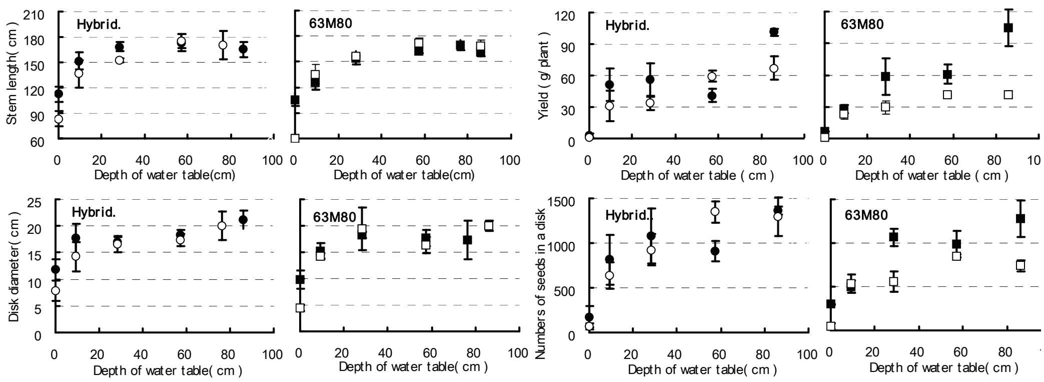

Fig. 5. Growth and yields on the sloping field.
Sown on June 7.●Hybrid. (Trad.) ■63M80 (NuSun)
Sown on June 26.○Hybrid. (Trad.) □ 63M80 (NuSun)

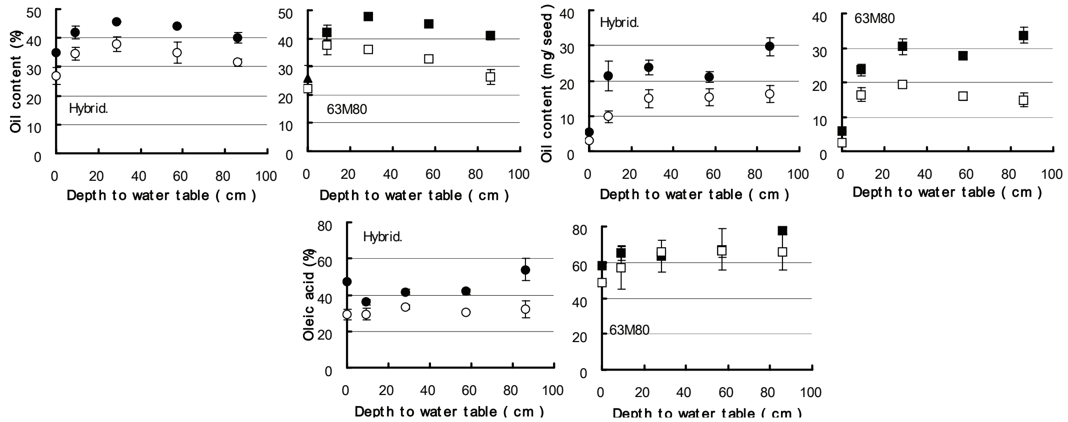

Fig. 6. Harvest quality on the sloping field.
Sown on June 7.●Hybrid. (Trad.) ■63M80 (NuSun)
Sown on June 26.○Hybrid. (Trad.) □ 63M80 (NuSun)

DISCUSSION

The increase in oleic/linoleic acid ratio and the decrease in oil content with increasing temperatures during grain filling have been widely reported. Izquierdo et al. (2006) reported that increasing night temperature resulted in higher oleic acid concentration, and Flagella et al. (2002) reported that a decrease in the oleic/linoleic acid composition ratio was observed in early sowing treatments (lower mean temperature) and under irrigation. In this study, a similar tendency was shown so that the decrease in oleic acid composition and the increase in linoleic acid composition were observed at lower temperature conditions and rise of water table. The oil content (%) was highest at the water table of 30 cm. With a deeper water-table the oil content was decreased. Oil content per one-gram seeds reached the highest at the same water-table depth. After that it remained unchanged. The oil accumulation in seed was presumably most active at around 30cm depth of water table, a comparatively shallow depth. In the case of soybean, Shimada et al. (1995) reported that the fluctuation of the water table reduced the yield. In this experiment, the water table condition was constant, which might have affected the results that even when the water table was shallow, around 30cm in depth, the oil content was the highest.

As a further step, it is necessary to gain a deeper insight into the physiological mechanisms behind the effect of excess moisture in order to increase seed yield and improve the harvest quality.

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Optimizing of potassium and magnesium fertilizers in sunflower production

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ABSTRACT
A factorial experiment with a randomized complete block design (RCBD) with three replications was carried out during 2001 at Khoy Agricultural Research Station to evaluate the main and interactive effects of potassium and magnesium on the yield and quality of Golshid variety of sunflower (*Helianthus annuus* L.) crop with due considerations to the antagonistic effects between these two nutrients in the context of determining the optimum levels of fertilizer application for the best yields as is often the main objective of plant nutrition research. The treatments included four levels of potassium (K_0=0, K_1=45, K_2=90 and K_3=135 kg K_2O as potassium sulfate per hectare) and four levels of magnesium sulfate (Mg_0=0, Mg_1=50, Mg_2=100 and Mg_3=150 kg magnesium sulfate per hectare). The results showed that the sunflower seed yield increased with increasing levels of potassium up to K_3=1.5 times the SWRI′s recommended rate which, combined with magnesium sulfate at a rate of 100 kg ha^{-1}, yielded the best with no significant increases at higher rates. The best rate for magnesium sulfate turned out to be 50 kg ha^{-1}. The application of potassium and magnesium increased thousand seed weight but no effect was seen on plant height or the diameter of the plant stem. The diameter of the sunflower disc improved with the application of potassium and magnesium but the effect was not significant. However, the best seed yield was obtained with the treatment K_2Mg_1 and the best disc diameter and the weight of a thousand seeds index were obtained with the treatment K_3Mg_2. Finally, the best ratio for the rates of potassium to magnesium was determined to be about 3.

Keywords: magnesium - potassium - seed yield - sunflower

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the high nutrient demanding plants (Glas and Kassel, 1988; Lie, 1996; Sepehr and Malakouti, 2002), often responding positively to the application of chemical fertilizers; however, despite the fact that there is a good potential for high sunflower yields in Iran, its cultivation in poor. Calcareous soils along with poor fertilizer programs (imbalanced use of fertilizers) have produced low yields causing too little interest in cultivation of this needed oil crop even though some 90% of the vegetable oil must be imported into the country. During the recent years, numerous attempts have been made to increase oilseed yields through fertilizers (Tandon, 1990; Amnuaysilpa et al., 1991; Shinde et al., 1993; Anadurai and Palaniappan, 1994; Krishnamurthi and Marthan, 1996; Malakouti and Sepehr, 2003), but few studies have been carried out with regard to Mg and its interaction with K in Iran. Therefore, the objective of this research was to evaluate the effect of K and Mg and their interactions on the yield of sunflower, and to determine optimal ratios of these nutrients for obtaining maximum yields.

MATERIALS AND METHODS

A factorial field study in a randomized complete block design (RCBD) with three replications was carried out during 2001 at Khoy Agricultural Research Station to evaluate the main and interactive effects of potassium and magnesium on the yield and quality of Golshid variety of sunflower (*Helianthus annuus* L.) crop with due considerations to the antagonistic effects between these two nutrients in the context of determining the optimum levels of fertilizer application for the best yields, often the main objective of plant nutrition research. Soil was clay-loam, low in organic matter, with a pH=8.1, and a total neutralizing value (TNV) of 14.8%. Extractable K and Mg were 190 and 440 mg/kg of soil, respectively. The treatments included four levels of potassium (K_0=0, K_1=45, K_2=90 and K_3=135 kg K_2O as potassium sulfate per hectare) and four levels of magnesium sulfate (Mg_0=0, Mg_1=50, Mg_2=100 and Mg_3=150 kg magnesium sulfate per hectare). Other fertilizers were applied at uniform rates of 350 kg ha^{-1} urea-N, 100 kg P_2O_5.ha^{-1} as triple super phosphate (TSP), iron sulphate (80 kg ha^{-1}), zinc sulphate (40 kg ha^{-1}), manganese sulphate (40 kg ha^{-1}), and boric acid (20 kg ha^{-1}) on all plots.

RESULTS

The results showed that the sunflower seed yield increased with increasing levels of potassium up to the rate of K_3, which is 1.5 times the SWRI′s recommended rate. When combined with magnesium sulfate, outyields of up to 100 kg ha^{-1} were reached, with no significant increases at higher rates (Table 1 and 2), The best rate for magnesium sulfate turned out to be 50kg ha^{-1} (Table 2). The application of potassium and magnesium increased the thousand seed weight, but no effect was seen on the plant height or the diameter of the plant stem (Table 1 and 2). The diameter of the sunflower disc improved with the application of potassium and magnesium, but the effect was not significant (Tables 1 and 2). However, the best seed yield was obtained with the treatment K_2Mg_1 and the best disc diameter and the weight of a thousand seeds index were obtained with the treatment K_3Mg_2 (Fig.).

Table 1. The effect of K rates on the seed yield, 1000-seed weight, and head diameter

Treatments	Rates (kg K_2O.ha^{-1})	Seed yield (kg ha^{-1})	1000-seed weight (g)	Head diameter (cm)
K0	0	3417 b	69.33 b	19.94 a
K1	45	3748 ab	71.20 ab	20.05 a
K2	90	3869 a	72.75 a	20.08 a
K3	135	3939 a	72.83 a	20.98 a

Table 2. The effect of Mg rates on the seed yield, 1000-seed weight, and head diameter

Treatments	Rates (kg $MgSO_4$.ha^{-1})	Seed yield (kg ha^{-1})	1000-seed weight (g)	Head diameter (cm)
Mg0	0	3500 b	69.88 b	19.67 a
Mg1	50	3948 a	71.17 ab	20.23 a
Mg2	100	3928 a	71.83 ab	20.93 a
Mg3	150	3597 ab	73.31 a	20.22 a

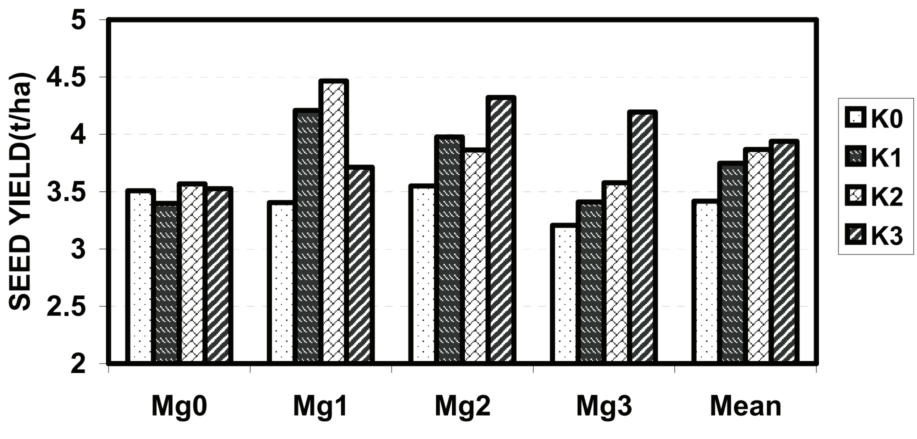

Fig. 1. The effects of potassium and magnesium on the seed yield.

DISCUSSION

According to this study, in order to obtain high yields, potassium and magnesium fertilizers must be applied in the right proportions, and the best ratio for the rates of potassium to magnesium was measured to be 3 for sunflower production in the Khoy area. Although the soils of the experiment site contained high levels of Mg, more than the total sunflower uptake during the growing season, since most of this nutrient (90%) was absorbed in the short period of flowering (Glas and Kassel, 1988), the rate of supply by the soil was not enough to meet the plant demands. Besides, our method of measuring soil Mg by acetate may not be accurate in estimating plant available Mg since some of the soil Mg may be in the form of insoluble salts like $MgCO_3$, not available to plants but extractable with acetate.

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Sunflower response to mineral nitrogen, organic and bio-fertilizers under two different levels of salinity

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ABSTRACT

This investigation was carried out during the two summer growing seasons of 2005 and 2006 at two locations in the north-east of the delta of Egypt. The first site (S1) is characterized by a good clay soil with fresh irrigated water while the second site (S2) has a salt-affected soil and is irrigated with a mixture of fresh and drained water. Two open pollinated cultivars of sunflower (Sakha 53 and Giza 102) were used. Seven different packages of the combinations of bio (cerealin), organic and mineral nitrogen fertilizers were used as follows: T1 is the recommended chemical nitrogen fertilize (45 Kg N/fad) (fad= Faddan = 4,200 m^2), T2 (1/2 N +30 m^3/fad of organic fertilizer) , T3 (bio fertilizer + 1/2 T1), T4 (bio fertilizer + 30 m^3 /fad organic fertilizer + 1/2 of T1), T5 (bio fertilizer + 30 m^3/fad organic fertilizer + 1/4 of T1), T6 (30 m^3 /fad of organic fertilizer + 1/2 of T1) and T7 (bio fertilizer + 30 m^3 /fad organic fertilizer). The results obtained showed that the application of farmyard manure in T4 has increased yield and yield component traits compared with the control treatment at S1 location. Head diameter, number of seeds per head, 100 seed weight, seed yield per plant and seed yield per plot were increased by 3.4, 13.4, 4.7, 12.8 and 16.8%, respectively, compared with the control treatment. T5 recorded the next rate of increase for the same traits by 2.2, 5.8, 5.8, 8.7 and 11.2%, respectively. The application of mineral nitrogen or organic manure has increased protein content in the good soil, while the mineral nitrogen alone (45kg N/fad) surpassed all other treatment in the salt-affected soil.

Key words: bio-fertilizer – mineral nitrogen – organic fertilizer – salinity

INTRODUCTION

Sunflower (*Helianthus annuus* L.) was chosen in this investigation as it is considered to be one of the most important promising oil crops in Egypt and it could be successfully grown in a great range of climatic conditions and soils. It could also play an important role in the cultivation of the new reclaimed lands, which are suffering drought, high temperatures and salinity effects. Organic and bio fertilizers were studied in this investigation as a replacement of part of the chemical nitrogen to reduce the total cost of cultivation and the chemical nitrogen pollution, and to improve the soil physical and chemical structure.

Singh et al. (1995) pointed out that oil content in sunflower seeds was reduced as the nitrogen increased from 40 to 80 kg N/ha. Singh et al. (1998) studied the content and uptake of nutrients by the sunflower crop as affected by *Azotobacter*, farmyard manure and NP levels. They showed that application of farmyard manure at 10 ton/ha, significantly improved the nitrogen and phosphorus contents in seed in both seasons and potassium content in second year only. In addition, they reported that the seed yield of sunflower was significantly higher with the farmyard manure (FYM) than with no FYM and *Azotobacter* inoculation treatments. El-Bana (2000) found a significant increase in oil yield per faddan (fad= Faddan = 4200 m^2) caused by the addition of organic matter and bio fertilizer (Cerealin). The interaction between organic matter application and inoculation with cerealin significantly increased seed oil content. Abou-Khadrah et al. (2002) pointed out that 100-seed weight, seed yield/plant and seed yield/fad were significantly increased by increasing nitrogen levels up to 45 kg N/fad.

MATERIALS AND METHODS

This investigation was carried out during the two summer growing seasons of 2005 and 2006 at Gamalia Dakahlia and at EL-Serw Agricultural Research Station in north east of the delta of Egypt. The first site (S1) has a good clay soil, while the second one (S2) is characterized by a salt-affected soil which is irrigated with a mixture of fresh and agricultural drained water.

Seven different packages of combinations of bio fertilizer, as cerealin, organic fertilizer as a farmyard manure (FYM) and mineral nitrogen (N) fertilizer besides the recommended rate of nitrogen were used as follows: T1 (45 Kg N/fad as the recommended chemical nitrogen fertilizer), T2 (30 m^3/fad FYM +1/2 T1), T3 (Bio fertilizer +1/2T1), T4 (Bio fertilizer +30 m^3/fad FYM + 1/2T1), T5 (Bio fertilizer +30 m^3/fad FYM + 1/4T1), T6 (15 m^3 /fad FYM + 1/2T1) and T7 (Bio fertilizer + 30 m^3 /fad FYM) (fad= Faddan = 4200 m^2).

The nitrogen fertilizer used, was a form of ammonium nitrate (33.3% N). The analysis of the farmyard manure (FYM), which was used as an organic fertilizer during both seasons (2005 and 20006), were: Moisture = 30%, 34 % ;C/N ratio = 11.92, 12.04; Organic matter = 10.40, 10.63 %; N = 0.51, 0.58%; P = 0.30, 0.27%; K = 3.74, 3.96 %; EC dS/m = 3.12, 3.27 and PH = 7.51, 8.04, respectively.

The soil salinity was 832 and 858 ppm at the site of Gamalia (S1; conventional soil) in both seasons, respectively, while at EL-Serw Agric. Research Station (S2; saline-affected soil) it was of 3140 and 3789 ppm, respectively. The electric conductivity of the irrigated water for S1 was 0.40 and 0.37 dS/m, while in S2 it was 1.60 and 1.51 dS/m for the first and the second seasons, respectively.

Seven random plants from the inner rows of each sub plot were taken at harvest time to determine plant height, number of leaves per plant, stem diameter, head diameter, number of seeds per head, 100-seed weight and seed yield per plant and the whole seed yield per plot were recorded.

The experiment plot contained 4 ridges (0.60 m width x 4 m long) and seeds were sown in hills (30 cm apart) on one side of each ridge and surface irrigation was used.

All data were subjected to the appropriate statistical analysis of variance as outlined by Snedecor and Cochran (1980). Data of the two seasons were compared by using the Least Significant Difference Test (LSD).

RESULTS AND DISSCUSION

The means of vegetative growth traits, yield and yield components, and oil and protein contents of the two varieties of the combined data over the two locations are presented in Tables 1, 2 and 3.

Vegetative growth traits

At the first site (S1), which was characterized with good soil, data obtained (Table 1) indicated that plant height under the fertilization treatment T4 showed a significant superiority over the control treatment (45 kg N/fad) and, to different extents, over the other treatments. The tallest plant (236 cm) was recorded with the higher FYM application (T4 treatment) followed by T5 treatment and the shortest plants were obtained with T7. On the other hand, at El-Serw site (S2), it was found that, with the exception of T2 treatment, plant height was significantly less under all fertilization treatments than under the control (T1) treatment. Data also revealed that the number of leaves/plant was significantly affected by all treatments at site S1 only. The highest and lowest values were observed with T4 and T7, respectively, while, there was no significant effect at El-Serw site (S2). These results indicate that the differences between the treatments were not great enough to reach the significant level. Stem diameters of plants were also significantly affected at both locations. T4 treatment recorded the highest value for this trait (Table 1) and showed a significant superiority over the other treatments tested. In contrast, the treatment consisting of the sole mineral nitrogen was the superior one in the second site (S2). These results could be attributed to the negative effects of salinity on the bio and organic fertilizers. At Gamalia (S1), data presented in Table 1 indicated that the highest number of days to 50 % flowering was recorded under the fertilization treatment T4, while T7 (bio. + 30 m^3 OM) recorded the lowest number. In contrast, at El-Serw (S2) location was observed the lowest number of days to 50% flowering (53.9) under the fertilization treatment T7. This means that the response of this trait to the seven selected fertilization treatments at the two locations was not the same and revealed contradictory findings.

Yield and yield component traits

The results presented in Table 2 showed that at Gamalia (S1) the application of organic manure and/ or cerealine to this good soil significantly increased head diameter, number of seeds per head, seed yield per plant and per plot. These increases were more pronounced under T4, but the absence of bio or organic fertilizers reduced the values of the mentioned traits in relation to the control treatment as shown in T2 or T3. Moreover, the rate of these two fertilizers alone gave the lowest values of the same traits and could not compensate for the absence of mineral nitrogen (45kg N/fad). These results indicate that the used rates of the bio and organic fertilizers did not meet sunflower requirement of nitrogen. The data also showed that the highest value of 100 seed weight was obtained under T5 treatments, in which the rate of the mineral nitrogen was reduced, whereas at S2, the T1 (45 kg N/fad) was superior in all studied traits except for the 100 seed weight.

Table 1. Effect of selected fertilization treatments on some vegetative growth traits at Gamalia and El-Serw locations (combined analysis of the two seasons).

Treatments	Plant height cm.		leaves /plant (N0)		Stem diameter(cm)		Days to flowering	
	S1	S2	S1	S2	S1	S2	S1	S2
T1 (45 kg N/fad)	227	126	31.1	27.2	2.22	1.55	56.8	55.7
T2 (1/2 N + 30 m^3 fym)	222	126	31.3	26.4	2.24	1.50	56.8	54.6
T3 (1/2 N. +30m^3 fym)	224	119	31.0	26.4	2.36	1.49	55.5	56.1
T4 (1/2 T1 + bio + 30 m^3org.)	236.	116	33.6	25.0	2.50	1.43	57.8	53.9
T5 (1/4 N + 30 m^3 fym)	228	118	31.8	24.8	2.38	1.21	56.2	56.6
T6 (1/2 T1 + 15 m^3 fym)	225	114	30.9	27.2	2.41	1.32	56.8	54.6
T7 (bio. + 30 m^3 fym)	212	117	30.7	26.6	2.27	1.40	55.3	56.8
L.S.D. $_{0.05}$	6.1	5.9	1.57	-	0.211	0.149	0.684	1.01

S1 = The good clay soil at Gammalia; S2 = The salt-affected soil at El-Serw

Table 2. Combined data of 2005 and 2006 seasons on some yield and yield component traits under different fertilization treatments.

Treatments	Head diameter (cm)		Number of seeds/head		100 seed weight (gm)		Seed yield /plant (gm)		Seed yield /Plot (kg)	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
T1 (45 kg N/fad)	22.0	17.4	1119.2	982.0	6.85	6.93	80.0	67.5	5.33	4.44
T2 (1/2 N + 30 m^3 fym)	21.4	17.2	1170.8	870.0	7.13	6.88	85.8	62.2	5.71	4.26
T3 (1/2 N. +30m^3 fym)	21.6	16.6	1157.3	875.2	6.91	5.79	83.2	53.1	5.69	3.65
T4 (1/2 T1+ bio+30 m^3org)	22.7	16.7	1269.2	893.3	7.17	6.53	90.2	60.6	6.22	4.02
T5 (1/4N+30 m^3 fym)	22.5	16.6	1188.2	856.7	7.27	6.53	87.6	57.1	6.00	3.79
T6 (1/2 T1+ 15 m^3 fym)	21.3	16.8	1156.2	862.5	6.89	6.27	83.2	56.6	5.65	3.75
T7 (bio. + 30 m^3 fym)	20.1	16.4	967.1	738.8	6.69	6.77	66.6	515	4.53	3.55
L.S.D. $_{0.05}$	1.01	0.68	62.8	62.6	0.562	0.359	4.74	3.92	0.151	0.164

Table 3. Combined data of 2005 and 2006 seasons on some quality traits under different fertilization treatments at Gamalia and El-Serw locations

Treatments	Seed oil (%)		Seed protein (%)	
	S1	S2	S1	S2
T1 (45 kg N/fad)	43.1	41.1	16.5	16.3
T2 (1/2 N + 30 m^3 fym)	43.3	40.2	16.9	15.9
T3 (1/2 N. +30m^3 fym)	42.2	41.4	16.1	15.6
T4 (1/2 N + bio.+30m^3fym)	43.0	40.6	17.0	16.3
T5 (1/4N+30 m^3org	41.7	39.9	16.1	16.1
T6 (1/2 N. + 15 m^3 fym)	41.3	42.9	16.0	15.8
T7 (bio. + 30 m^3 fym)	42.5	41.2	17.2	16.6
L.S.D. $_{0.05}$	0.611	0.524	0.416	0.418

The results indicated that the stimulatory effect of the combination of the three fertilizer sources, i.e mineral nitrogen, FYM and cerealine at site 1 in the previous treatments on the seed yield and yield components traits may be attributed to increasing the meristemic and enzymatic activities which encourage plant growth. Meanwhile, the release of N of the FYM and cerealine were not enough to compensate for the 50% reduction of chemical nitrogen dosage in S2 location. The salinity may also negatively affect the microorganism activity For this reason, the full dosage of N (45kg N/fad) was superior in the salty soil. These results are in agreement with those obtained by Singh et al. (1998) and Abou-Khadrah et al. (2002).

Quality traits

The results at S1, presented in Table 3, indicated that most seed oil percentages obtained under fertilizer treatments were lower than those obtained under control treatment T1, especially T4 and T6 by 0.2 and

4.2%, respectively, while T2 showed an increment over the control treatment by 0.4%. At site2, data also indicated that seed oil percentage under treatments T6, T3 and T7 showed an increase over the control treatment which was 4.5, 0.8 and 0.4% respectively. However, for fertilizer treatments T4 and T5 oil contents were lower than the obtained under control treatment T1 by 1.0 and 2.9%, respectively. These results indicated that the highest nitrogen fixation gave the highest protein content and the lowest oil content at site 1.

These results may be due to the effect of organic manure by improving the physical structure of the soil and increasing available nitrogen, which reflects the greater growth and, consequently, more absorption of nitrogen and more crude protein synthesis. These results are in line with those obtained by El-Bana (2000) and El-Sadek (2005).

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The effect of different amounts of animal manure on qualitative and quantitative traits of sunflower hybrid varieties

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ABSTRACT

A field study was conducted to investigate the effect of different amounts of animal manure on quantitative and qualitative traits of sunflower hybrid varieties in 2006-2007 at the Uromia Agricultural Research Station, Iran. The experimental design was a completely randomized block with three replications. The first factor was animal manure at 0, 15, 30 and 45 t ha^{-1} and the second factor was 3 hybrid varieties: Euroflir, Alistar and Golshid. The parameters assessed were head diameter, stem diameter, number of seeds per head, 100 seed weight, oil percentage, protein percentage, harvest index, seed yield, biomass and oil yield. Results of this study showed significant effects of different amounts of animal manure and various varieties on head diameter, number of seeds per head, 100 seed weight, seed yield, and oil yield. Comparison of mean data showed that by increasing rate of manure, the head and stem diameter, number of seeds per head, 100 seed weight, seed yield, biomass and oil yield were increased. The results revealed that the Golshid variety had the highest values for all the traits, except oil percentage and harvest index. Correlation analysis showed that seed yield had a significant positive correlation with head diameter, stem diameter, plant height, number of seeds per head, 100-seed weight, protein percentage, biomass and oil yield. According to these results, application of 45 t ha^{-1} animal manure increases the quantitative and qualitative yield of sunflower. Golshid variety was found to be suitable for cultivation in the region.

Key words: animal manure – hybrid varieties – oil yield – seed yield – sunflower.

INTRODUCTION

Sunflower has modest fertility needs, but does respond to animal manure. When following soybeans in the rotation, roughly 30 to 50 t of animal manure per ha are appropriate. Following a non-legume, about 80 to 100 t of animal manure per ha is suitable. Animal manure or a legume cover crop can reduce or eliminate need for N fertilizer (Khajehpour, 1998).

Sunflower (*Helianthus annuus* L.) has relatively proved to be a good oil seed crop in Iran. It is a potential source of high quality edible oil. Due to the increasing edible use of this oil crop, its production has been enhanced rapidly all over the world. Sunflower seed contains 48-52% of good quality edible oil and 40-50% of protein in the meal (Khajehpour, 1998). The oil cake from sunflower is also useful for cow and fish feeding. At present, sunflower is grown in many districts of Iran without proper care. The total cultivation area of this oil crop is limited. The progress in sunflower production has been slow due to the lack of proper production technologies and management practices. Among the several agro-techniques which can enhance the production of yield is the use of proper land preparation, irrigation, fertilizer application, proper plant spacing and other important related factors. So, an attempt has been made to study the effect of animal manure for obtaining a maximum yield of sunflower.

MATERIALS AND METHODS

The experiment was conducted in the experimental field of Uromia Agricultural Research Station, Iran in 2005. The soil of the experimental site was loamy, pH of 8.5, 0.9% organic matter and 0.09% total nitrogen. The unit plot size was 3 by 5 m. The varieties used for the study were 3 hybrid varieties: Euroflir, Alistar and Golshid. The experiment was carried out in a randomized block design with three replications. The row spacing was 60 m. The plant spacing was 25cm. With regard to the results of the animal manure analyses under study, the concentrations of total N, P and K were 1.54, 0.75 and 2.8% respectively. At the time of land preparation, animal manure was incorporated into the soil. At harvest time, 10 plants were selected randomly from each plot and plant height and different yield contributing characters (Table 1) were measured. Grain protein content was also determined by a grain analyzer. The data were analyzed using the SAS statistical package (SAS Institute, 1996) and the mean comparisons were made following Duncan's multiple range test at P = 0.05 by MSTATC (version 2.10, Inc, Michigan State University). The correlation coefficients between all pairs of traits were determined by the SPSS statistical package (version 10, Chicago, USA).

RESULTS AND DISCUSSION

The data on the effect of the animal manure and the cultivar and their interaction are presented in Table 1. By increasing the rate of animal manure, the head diameter was increased. The maximum head diameter was obtained from 30 t ha^{-1} treated plots. Comparison of different cultivars showed that Golshid cultivar had the highest head diameter at any of the animal manure levels. This result is consistent with previous reports that plant growth, photosynthesis and nutrient uptake are affected (increased) under animal manure treatments (Kandil et al., 1988). Animal manure had also a significant effect ($p \leq 0.05$) on stem diameter, number of seeds per head, 100 seed weight, seed yield, biomass, and oil yield of all cultivars (Table 1). The response of the cultivars differed significantly with increasing animal manure levels ($p \leq 0.05$). Golshid cultivar had the highest value of the above measured parameters at all animal manure rates.

Table 1. Analysis of variance to test the effect of animal manure on yield and yield contributing traits of sunflower[1]

Source	d.f.	Head Ø	Stem Ø	Plant height	Number of seeds per head	100 seed weight	Seed yield	Bio-mass	Harvest index	Oil content	Protein content	Oil yield
Replication	2	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	*
Animal manure	3	**	*	ns	**	**	**	**	ns	ns	ns	**
Cultivar	2	**	**	**	**	**	**	**	**	ns	**	**
Animal manure x cultivar	6	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	*

[1]ns, not significant; *, ** Significant at 0. 05 and 0. 01 probability levels, respectively.

With increasing of animal manure levels, the sunflower stem diameter increased, which may be due to increasing nutrient uptake and translocation and increasing of photosynthesis (Hassanzadeh-Gorttapeh et al., 2006).

In crop production, the most important part is the plant's reproductive stage. If photoassimilates are allocated at a suitable time, the number of filled seed per head increases. Although the important yield components are seed weight and number of seeds per head, heads with a large diameter accompanied by more seeds could result in giving a higher yield (Vannozzi et al., 1987).

The maximum number of seeds per head was found by application of animal manure at 45 t/ha. The results were in close conformity with those of Steer et al. (1984). In general, the application of animal manure improved the yield components of sunflower. The plots treated with 45 t/ha animal manure produced the highest 100 seed weight. The results are in close agreement with the findings of Hassanzadeh-Gorttapeh et al. (2006).

According to the results of this study, we can conclude that the application of animal manure can reduce the input consumption per unit area. Among the cultivars studied, Golshid cultivar gave the highest yield due to its higher head weight, number of seeds per head and 100 seed weight. No significant difference in the oil content among the cultivars studied was observed. Therefore, Gholshid cultivar is suitable and may be recommended for this region. The results are in close agreement with the findings of Ulger et al. (1993) and Singh et al. (1996).

Correlation coefficients between traits are presented in Table 2. As reflected in the literature, the number of seeds per head is the yield component most significantly correlated with grain and oil yield (Connor et al., 1997; López-Pereira et al., 1999; Steer et al., 1984). Moreover, the stronger correlation between grain yield with oil yield, compared with that between oil percentage and oil yield, shows that ultimately higher grain yields will mean higher oil yield for farmers. The highly positive correlation ($r=0.87^{**}$) between grain yield and plant height (Table 2) suggest that the seed yield is positively influenced by biomass, in addition to the number of seeds per head (Kesteloot, 1982; Lakshmanrao, 1985).

Table 2. Correlation coefficients between sunflower traits under treatment with different doses of animal manure[1]

	Head Ø	Stem Ø	Plant height	Number of seeds per head	100 seed weight	Seed yield	Bio-mass	Harvest index	Oil content	Protein content
Oil yield	0.78**	0.81**	0.81**	0.94**	0.83**	0.97**	0.92**	0.72**	0.27 ns	0.40*
Protein content	-0.38*	0.52**	0.46**	0.20ns	0.75**	0.50**	0. 53**	-0.55**	-0.55**	
Oil content	-0.45**	-0.48**	-0.39**	-0.31ns	-0.60**	-0.48**	-0.49**	0.40*		
Harvest index	-0.81**	-0.89**	-0.89**	0.61**	0.76**	0.55**-	-0.88**			
Biomass	0.48**	0.91**	0.89**	0.85**	0.89**	0.96**				
Seed yield	0.81**	0.85**	0.83**	0.92**	0.90**					
100 seed weight	0.77**	0.84**	0.79**	0.69**						
Number of seeds per head	0.78 **	0.77**	0.77**							
Plant height	0.88 **	0.96**								
Stem diameter	0.96 **									

[1]ns, not significant; *, ** Significant at 0. 05 and 0. 01 probability levels, respectively.

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Nitrogen fertilization of high oleic sunflower in wet climate

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ABSTRACT

Fertilization may help crops to yield better. To determine whether meteorological and soil conditions influence the productive response of nitrogen fertilization in sunflower a study was conducted in an Italian interregional project (BIOLI). The effects of nitrogen fertilization on two commercial high oleic varieties (Carnia and PR 64 H 61) were investigated in North East (Udine) Italy in 2005-2006-2007. Nitrogen fertilization gave the best yield at the highest level in Udine in wet and irrigated conditions. In Udine the locally selected high oleic hybrid (Carnia) had the best performance. Nitrogen fertilization is suggested only in good weather conditions and in nitrogen-poor soil. Under drought conditions nitrogen influences plant growth but not yield.

Key words: fertilization – irrigation condition – nitrogen – soil condition – sunflower.

INTRODUCTION

As in other crops, sunflower requires NPK fertilization. In Italy, trials with potassium (K) and phosphorus (P) in the last decade did not show any response in the crop due to the naturally high level of potassium in the soil, at least 160 mg/kg of available K_2O (international method), or due to the large quantity of fertilizer applied, in the effort to build up phosphorus levels. For phosphorus, the levels above 10-20 mg/kg of P_2O_5 in the soil (Olsen method) are maintained by annually applying the amount that was removed by the previous crop. In addition, sunflower has only moderate phosphorus requirements and utilizes mycorrihizas (Glass, 1988).

Nitrogen fertilization is very variable and depends on the amount of the element already present in the soil and the potential yield of the environment. Crnobarac et al. (2004) and Monotti (1978) reported that 100 kg/ha was suitable. Malligawad et al. (2004) expressed the importance of nitrogen combined with phosphorus and potassium and reported better yields when the ratio of the first two elements was between 1.5 and 2.0 (results of two experiments). Steer et al., (1994) reported that sunflower has a high nitrogen requirement. Bonari et al. (1992) associated the needs for nitrogen with available water. Laureti and Pieri (1999, 2001) reported that 40-80 kg/ha (depending on the water available) of fertilizer alone or associated with green manuring was enough. Moreover, according to Merrien et al. (1986), the nitrogen of the soil participates by up to 70% in the plant nutrition and is adsorbed particularly from 40th to 80th days from emergence. When flowering starts (60 days after emergence) the 50% of the nitrogen adsorbed is in the leaf. After that, nitrogen moves in the head and finally in the seeds. The coefficient of nitrogen fertilizer utilization in sunflower is 20-30% (60% in wheat) and that coming from fertilizers is adsorbed starting from flowering.

In an effort to contribute to the debate, under an interregional project, three levels of nitrogen were tested.

MATERIALS AND METHODS

To study the response of two high oleic sunflower hybrids (Carnia and PR 64 H 61) against nitrogen fertilization, three different levels of N (0; 60; 100 kg/ha) were used in two field experiments in two locations during 2005-2006-2007, under irrigated conditions at Udine, North East Italy. The experiments were laid in a randomized complete block design with four replicates with an individual plot size of 279 m^2 (9 x 31 m).

Weather conditions (temperature and rainfall) observed during the experiments are presented in Fig. 1. The average annual rainfall at Osimo is usually half that of Udine. In the experimental year the rainfall at Osimo was normal whereas, in May and June, the levels were below normal in Udine and was necessary to compensate with four irrigations of 30 mm each, every ten days starting from the 10th of May until the 10th June (May 10 and 20; June 1 and 10).

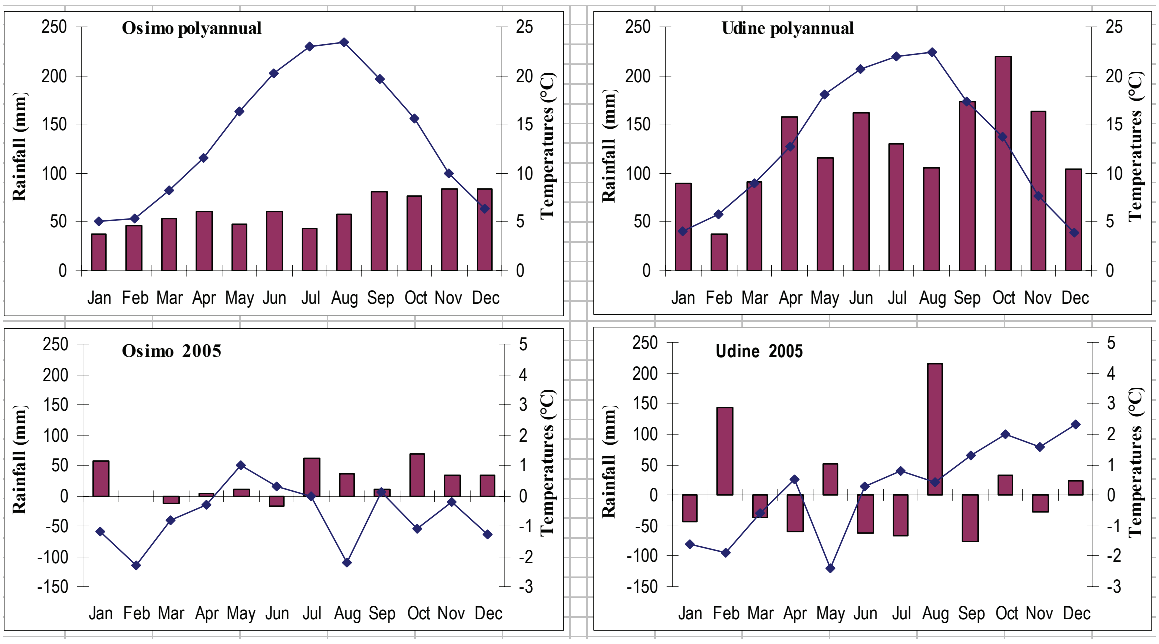

Fig. 1. Rainfall (mm) and mean temperatures (°C) in 2005 compared with the previous polyannual period of 20 years for Osimo and 10 years for Udine.

Soil tests showed high phosphorus and potash levels in both locations but low nitrogen content, especially in Udine (Table 1).

Table 1. Chemical properties of experiment field soils

	Osimo	Udine
Sand g/kg	133	400
silt g/kg	472	430
clay g/kg	395	170
nitrogen g/kg (N)	1.1	0.2
Phosphorus mg/kg (P)	11	41
potash mg/kg (K)	423	200

The soil was a Vertisol in Osimo with good water availability down to a deep level, whereas the soil was gravelly at Udine with good water availability only in the upper 50 cm and very poor water availability at deeper level. To satisfy crop water requirements, four irrigations (30 mm each) were done in May and June in Udine.

RESULTS AND DISCUSSION

Sunflower yield in Italy is greatly dependent on the amount of water stored in the soil and on the amount and distribution of rainfall during the vegetative period. In the summer of 2005 rainfall at Osimo, before blooming and seed filling, was below average so the yield was less than expected based on the plant size. In fact, during the whole cycle the better fertilized plots were always greener, taller and with larger leaves (Table 2). The only datum recorded for this aspect, plant height, was in fact influenced by nitrogen; the plants were taller with higher doses of nitrogen at both Osimo and Udine.

Table 2. Sunflower response to nitrogen fertilization

Nitrogen kg/ha	Yield t/ha		Oil content %		Oil yield t/ha		Thousand-seed weight g		Plant height cm
	Osimo	Udine	Carnia	PR64 H61	Osimo	Udine	Osimo	Udine	
0	2.14	2.27	47.4	48.1	0.91	1.01	62.3	48.2	165
60	2.24	2.27	49.0	47.8	0.96	1.02	62.8	47.6	173
100	2.24	3.28	45.8	47.9	0.94	1.42	62.6	55.6	178
LSD	0.31		1.3		0.14		4.07		4

The data recorded agree with those of Blanquet et al. (1987) who found a weak response whenever water availability was less than 200 mm during the crop cycle. In Udine, on the contrary, the highest nitrogen dose gave the best yield, but the intermediate dose (60 kg/ha) did not differ from the control (Table 2).

The highest yield was due to improved seed weight and number of seeds per plant. The positive response of nitrogen in Udine could be related to the very low nitrogen level in the soil. The improvement in Osimo was not evident because seed set was negatively influenced by the scarcity of rainfall during blooming; the subsequent good meteorological conditions of above average rainfall only produced an increased seed size.

The seed oil content changed as a function of fertilization only in Carnia (Table 2), whose value decreased at the highest nitrogen rate, but not in PR 64 H 61. Oil yield showed the same figures as seed yield, with the higher value only in Udine at the highest nitrogen fertilization.

In spite of good water availability the crop in Udine did not reach the same thousand seed weight (TSW) due to the large number of seeds set.

Yield differences were not observed in the hybrids used in the experiment at Osimo (Table 3) whereas at Udine the locally selected hybrid (Carnia) was significantly more productive than PR 64 H 61 probably due to its higher capacity to set seed. Carnia had also the best oil content at Udine and consequently the best oil yield, whereas at Osimo no differences were found.

Table 3. Variety differences

Varieties	Yield t/ha		Oil content %		Oil yield t/ha		Thousand-seed weight g		Plant height cm	
	Osimo	Udine	Osimo	Udine	Osimo	Udine	Osimo	Udine	Osimo	Udine
CARNIA	2.39	2.81	45.2	49.6	0.89	1.27	60.4	47.7	153	185
PR 64 H 61	2.24	2.17	48.3	47.5	0.99	1.07	64.7	53.2	177	175
LSD	0.25		1.1		0.11		4.07		5.0	

For plant height, PR 64 H 61 was little influenced by water availability, whereas Carnia was more sensitive

CONCLUSIONS

According to the literature, the response of sunflower to nitrogen fertilization is influenced by weather conditions during the season and the natural nitrogen level in the soil.

Under drought conditions and medium natural soil nitrogen content, the response of the crop was evident in the size of the plant but not in its yield. On the contrary, excessive growth could cause lower water use efficiency, but this was not evident in the trials.

Under good water conditions and low nitrogen content in the soil, sunflower responded positively to fertilization; the highest dose improved the amount of seed set, seed size, and, consequently, yield.

The results among the varieties tested were similar in Osimo and significantly different in Udine where the most productive local variety was used.

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Efficiency of modeling sunflower and *Amaranthus retroflexus* L. competition

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ABSTRACT

In order to evaluate the efficiency of empirical models of sunflower (*Helianthus annuus* L.) and redroot pigweed (*Amaranthus retroflexus* L.) competition, factorial experiments were established on randomized complete block design during 2005-2006. Treatments were three weed densities (8.3, 25 and 41.7 plants m^{-2}), three times of weed emergence (15 and 30 days after sunflower emergence) and three sunflower cultivars (Azarghol, Hysun and Allstar). Three weed-free sunflower plots were used as control. Yield was analyzed by three non-linear regression models. Results showed that in any sunflower cultivar, leaf area index (LAI) decreased significantly when weed density increased and redroot pigweed emerged with sunflower, and in full-season competition of 41.7 plants m^2, reduction of LAI in Allstar was two-fold compared with Azarghol. Reduction in Allstar LAI at interference with redroot pigweed took place earlier, compared with Azarghol and Hysun. Azarghol could tolerate 8.3 weeds/m^2 from 15 days and 41.7 weeds/m^2 from 30 days after sunflower emergence. In the short height cultivar Allstar, yield loss was higher than in Azarghol and Hysun. The model of Cousens (1985) was suitable for yield estimation of Hysun and Allstar, while the Model of Cousens et al. (1987) was the best description for the yield of Azarghol.

Key words: interference time – leaf area index – models of competition – redroot pigweed – sunflower – yield estimation.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oil crops, considerably affected by weed interference. Herbicide weed control is usually expensive. On the other side, redroot pigweed (*Amaranthus retroflexus* L.) is a one of the most troublesome weeds in sunflower in Iran.

The aim of weed management is changing of competitive relationships between weeds and crops (Aldrich, 1984). High competition power increases light interception efficiency (Assemat and Allirand, 1995). In McLachlan et al. (1993) opinion, the inhibitory effect of one plant species in relation to light received by another plant is a main factor in weed-crop competition modeling. As reported by Oliver et al. (1976), leaf area index (LAI) is a suitable physiological trait for evaluation of the amount of competition and production in plants. Between crop yield loss and weed density there is a sigmoidal relationship, which has an asymptote. So that, in low weed density, crop yield loss rate is lower, but with increasing of density, the rate of yield reduction increases, while at higher densities, because of high intra-specific competition between weed plants, yield loss rate decreases again (Beckett et al., 1988). Cousens et al. (1987), Kropff and Lotz (1992) and Cousens (1985) have used hyperbolic equations for modeling the relationships between weed density and crop yield loss. With the application of weed density and interference time-based models, the accuracy of the model will increase (Knezevic et al., 1997; Bosnic and Swanton, 1997). Cousens et al. (1987) developed hyperbolic equations in which crop yield is estimated in relation to density and relative interference time of weeds. Harper (1983) reported that some variables such as density, growth rate and emergence time of weeds are the most important factors in weed-crop competition for light interception.

The objective of this study was to evaluate sunflower vs. redroot pigweed competition using several empirical models previously developed.

MATERIALS AND METHODS

This study was conducted during 2005-2006 in Research Station of Tabriz University (Latitude 38º53'; Longitude 46°17', elevation 1360m), located in the north-west of Iran, with a semiarid and cold climate. The experimental design was a factorial combination of three weed densities (8.3, 25 and 41.7 plants m^{-2}), three times of weed emergence (15 and 30 days after sunflower emergence), and three sunflower cultivars

(Azarghol, Hysun 33 and Allstar RM). Three weed-free sunflower plots were used as control. The fertilizer used before planting was 150 kg ha^{-1} potassium sulfate, 150 kg ha^{-1} ammonium phosphate and 75 kg ha^{-1} urea. Data were analyzed with MSTAT-C software. Comparison of means was done using the Duncan's Multiple Range Test.

To determine the relationship of weed density and sunflower yield, an hyperbolic model (Equation 1) was used, as described by Cousens (1985):

YL=(I.d)/[1+(.Id)/A] where YL is yield loss of sunflower, d is weed density and I and A are coefficients of the model.

For determination of relationship between weed density and emergence time with sunflower yield the model of Cousens et al. (1987), was used (Equation 2):

YL= (I.d)/[c.t+(I.d)/A] where YL is yield loss of sunflower, d and c are weed density and emergence time and I, A and c are coefficients of the model.

One parameter model (Equation 3) was used for determination of relationship between sunflower yield loss and weed relative leaf area.

YL=(q.Lw)/[1+(q-1)Lw] where YL is yield loss of sunflower and q is coefficient of relative damage. Lw is relative leaf area of weed, which was calculated by equation 4.

$Lw=LAI_r/(LAI_s + LAI_r)$ where r and s indicate redroot pigweed and sunflower, respectively.

In order to evaluate the validity of abovementioned yield estimation models, four statistics were used, as follows (Thornley and Johnson, 1990):

Correlation between estimated and observed yield values (equation 5):

$$r = \frac{\sum_{i=1}^{n}\left(O_i - \overline{O}\right)\left(P_i - \overline{P}\right)}{\sqrt{\sum_{i=1}^{n}\left(O_i - \overline{O}\right)^2} \times \sqrt{\sum_{i=1}^{n}\left(P_i - \overline{P}\right)^2}}$$

where O_i=observed yield loss, $\overline{O}$ mean of observed yield loss, P_i estimated yield loss and P mean of estimated yield loss.

Mean Percentage Error (Equation 6):

$$MPE=\left[\sum_{i=1}^{n}\left(\frac{\left|obs_i - sim_i\right|}{obs_i}\right)\times 100\right]/n$$

Where obs_i and sim_i are observed yield and estimated yield, respectively, and n is number of treatments.

Root Mean Square Error (Equation 7):

$$RMSE = \left[(\sum_{i=1}^{n}\left(sim_i - obs_i\right)^2)/n\right]^{0.5}$$

Mean Bias Error (Equation 8):

$$MBE = \left[\sum_{i=1}^{n}\left(sim_i - obs_i\right)\right]/n$$

RESULTS AND DISCUSSION

In the three sunflower cultivars, LAI decreased significantly as weed density increased and redroot pigweed emerged simultaneously to sunflower, and in full-season competition of 41.7 plants m^{-2}, reduction of LAI in Allstar was double compared with Azarghol (Table 1). Also, in high densities and early time of weed emergence, LAI and canopy light transmission of redroot pigweed increased significantly, and canopy closure happened earlier (data not shown). In full season competition of 41.7

weeds/m^2, LAI in sunflower decreased from 4.57, 4.20 and 3.90 in the controls to 3.69, 3.12 and 2.56 in Azarghol, Hysun and Allstar, respectively (Table 1). In Allstar, weed density was more effective than weed interference time. For each day delaying weed emergence time, sunflower LAI in i_0-i_{15} and i_{15}-i_{30} decreased 180 and 160 cm^2 per unit area, respectively (Fig. 1). On the other hand, in spite of same interference time duration (15 days) in the above mentioned treatments, reduction value in LAI at i_0-i_{15} was higher than i_{15}-i_{30}, as reported by Knezevic et al. (1994). In that study, when redroot pigweed emerged with corn, LAI in corn decreased by 36%, but in delayed interference time (3-5 leaves stage of corn), LAI reduction value was not significant. It seems that, between physiological characteristics in crops, LAI is more effective in compatibility of crops and influences the amount of light interception by canopy and availability of weeds to light.

Studying the effect of weed density on sunflower LAI at 30-90 days after emergence, it was observed that the difference between redroot pigweed density levels in the three cultivars, especially in Allstar, starts from early growth stages, and difference value is gradually increased. Reduction in Allstar LAI happened earlier, compared with Azarghol and Hysun. In 45-75 days after sunflower emergence, increasing rates of sunflower LAI at i_0, i_{15} and i_{30} were 750, 810 and 850 cm^2/day in Azarghol, 630, 700 and 770 cm^2/day in Hysun, and 550, 590 and 600 cm^2/day in Allstar, respectively (Table 1). Negative growth of LAI in Allstar at interference with redroot pigweed was in advance from 75 DAE, compared with control. This condition which arose from leaf senescence resulting from weed shading, caused a LAI reduction of 410 cm^2/day at 80-85 DAE in Allstar cultivar, while the negative growth of LAI in the control had not yet started. As reported by Hall et al. (1992), Tollenaar et al. (1994), Knezevic et al. (1994), and Bosnic and Swanton (1997), LAI is one of the most important characteristics indicating the competition power of plants and could be used in estimation of crop yield loss at interference with weeds. With regard to the effect of LAI on plant photosynthesis and the effect on yield of the latter, it is expected that redroot pigweed causing a reduction of sunflower LAI, causes also a significant reduction in yield.

Increasing of redroot pigweed density increased weed LAI, but the increased value in delayed weed emergence time was reduced, especially in Azarghol and Hysun (Table 1). Therefore, when increased weed density from 8.3 to 41.7 plants/m^2 at i_0, weed LAI increased from 0.66 to 0.76 (13% increase) in Azarghol, from 0.9 to 1.06 (15% increase) in Hysun and from 1.01 to 1.19 (15% increase) in Allstar. But at i_{30}, the effect of a similar density increase on LAI was not significant in Azarghol, whereas it increased from 0.75 to 0.78 (4% increase) in Hysun and from 0.95 to 1.07 (19% increase) in Allstar. These results showed that the weed interference time was more effective than density in Azarghol and Hysun, but weed density was more effective than interference time in Allstar, and canopy condition was suitable for development of weed LAI in Allstar (Fig. 2).

The three studied cultivars indicated different interactions of densities and interference times of redroot pigweed with grain yield (Table 1). Azarghol could tolerate 8.3 weeds/m^2 from 15 DAE and 41.7 weeds/m^2 from 30 DAE, without significant reduction in yield. Redroot pigweed could decrease sunflower yield of Azarghol only at high densities (>25 weeds/m^2). In Hysun, any of the studied treatments could produce similar yield to the control plot. Significant difference in sunflower yield arising from early emergence time of redroot pigweed was expected, as weed interference time in relation to crops is a main factor in crop yield loss (Kropff et al., 1992; Rajcan and Swantom, 2001).

Allstar experimented higher yield loss compared to Azarghol and Hysun. This was explained on the basis of its shorter stature, which favored the competitive power of redroot pigweed with this cultivar. Knezevic et al. (1997) reported that yield loss values in tall and short sorghum cultivars at interference with redroot pigweed were 16% and 75%, respectively, because of higher LAI in the taller cultivar. In the present study, a higher oil yield was obtained from treatments with greater grain yield, and the lowest oil yield was observed in treatments of full season interference of 41.7 weeds/m2 in the three cultivars.

In Azarghol and Hysun hybrids, correlation coefficients between redroot pigweed relative leaf area and sunflower yield loss were 0.99 and 0.92 and distinction coefficients of the model were 0.41 and 0.75, respectively; Also, higher RMSE values (378.32 and 342.62, respectively) indicated that this model has a low efficiency in estimating yield loss in these two hybrids. We obtained similar results in Allstar (data not shown).

In the comparison of observed and estimated yield values by the model in Hysun and Allstar hybrids, it was observed that MBE, RMSE and MPE values were +0.003, 28.87 and 8.01%, respectively in Hysun, and +0.003, 41.47 and 4.64%, respectively in Allstar. This model was suitable for yield estimation of these two cultivars (Fig. 3, 4).

Correlation between observed and estimated yield values in Azarghol hybrid was 0.99. Values of MBE, RMSE and MPE were calculated as being +0.09, 18.58 and 2.16%, respectively. With regard to reduction in RMSE value in this model as compared with the model of Cousens (1985), it seems that, this model was the best description for the yield of Azarghol (Fig. 5). Besides, as reported by Bosnic and Swanton (1997), if SE values of model parameters were lower than half the parameter main value, the model has a higher validity for the estimation of crop yield. The accuracy of Cousens et al. (1987) model in the estimation of yield loss in Hysun and Allstar hybrids was lower.

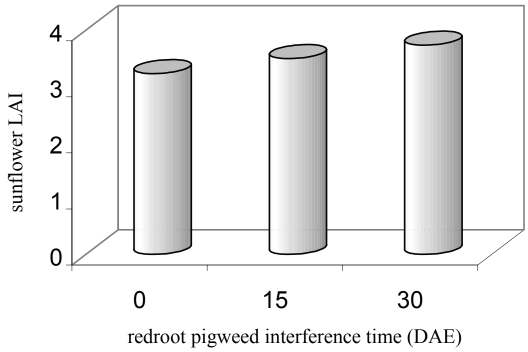

Fig. 1. Effect of redroot pigweed interference time on sunflower LAI at 90 days after emergence

Table 1. Mean comparisons for some of variables studied.

Treatments[1]	LAI of sunflower at 75 DAE[2]	LAI of redroot pigweedat 75 DAE[2]	Sunflower grain yield (kg/ha)[2]
$V_1D_1I_0$	4.07 k	0.66 w	3343 cd
$V_1D_1I_{15}$	4.35 e	0.57 z	3960 ab
$V_1D_1I_{30}$	4.49 b	0.53 z	4061 ab
$V_1D_2I_0$	3.96 i	0.72 v	3109 de
$V_1D_2I_{15}$	4.26 f	0.59 x	3884 b
$V_1D_2I_{30}$	4.39 c	0.55 z	4036 ab
$V_1D_3I_0$	3.69 q	0.76 t	2716 fg
$V_1D_3I_{15}$	4.09 i	0.61 x	3578 c
$V_1D_3I_{30}$	4.37 d	0.56 z	4081 ab
$V_2D_1I_0$	3.52 t	0.90 n	2151 h
$V_2D_1I_{15}$	3.91 m	0.80 q	2726 fg
$V_2D_1I_{30}$	4.11 h	0.75 u	3507 c
$V_2D_2I_0$	3.40 v	0.99 i	2032 hi
$V_2D_2I_{15}$	3.81 o	0.81 q	2556 g
$V_2D_2I_{30}$	4.08 j	0.77 r	3238 d
$V_2D_3I_0$	3.12 y	1.06 h	1722 j
$V_2D_3I_{15}$	3.40 v	0.85 o	2078 h
$V_2D_3I_{30}$	3.77 p	0.78 r	2960 ef
$V_3D_1I_0$	3.42 u	1.01 j	1581 j
$V_3D_1I_{15}$	3.58 s	0.95 m	1793 ij
$V_3D_1I_{30}$	3.62 r	0.90 n	2039 hi
$V_3D_2I_0$	2.92 z	1.09 f	830 lm
$V_3D_2I_{15}$	3.16 x	1.02 i	1032 kl
$V_3D_2I_{30}$	3.24 w	0.99 k	1226 k
$V_3D_3I_0$	2.56 z	1.19 c	535 n
$V_3D_3I_{15}$	2.80 z	1.11 e	651 mn
$V_3D_3I_{30}$	2.91 z	1.07 g	752 mn
V_1D_0 (control)	4.57 a	-	4171 a
V_2D_0 (control)	4.20 g	-	3858 b
V_3D_0 (control)	3.90 n	-	3153 de
LSD	0.005	0.2277	239.7

[1]V indicates sunflower variety; D and I indicate density (plants/m^2) and time of emergence (days after sunflower emergence) of redroot pigweed, respectively.
[2]Values within columns followed by the same letter have no significant difference at the 0.01 probability level.

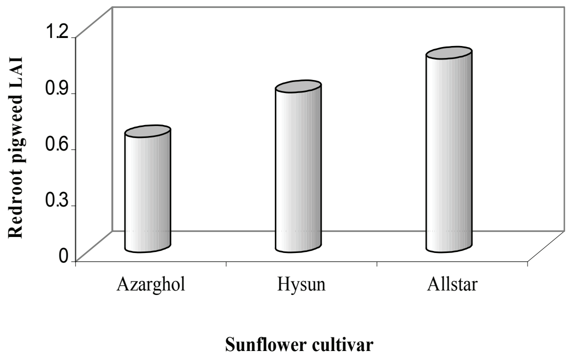

Fig. 2. Effect of sunflower cultivar on redroot pigweed LAI at 90 days after sunflower emergence

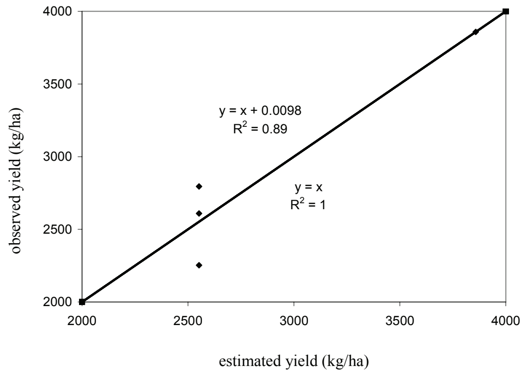

Fig. 3. Comparison of observed yield vs estimated yield by the model of Cousens (1985) at Hysun cultivar. YL=(1.1d)/[1+(1.1d)/33.84], where YL= estimated yield loss and d=weed density.

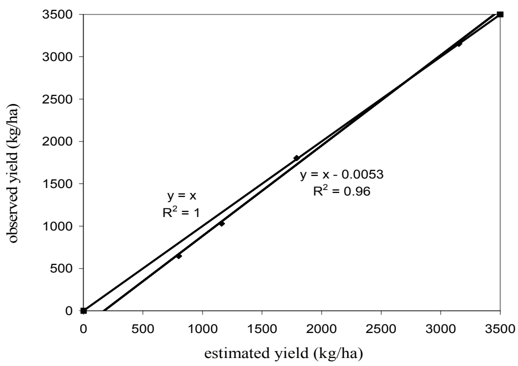

Fig. 4. Comparison of observed yield vs estimated yield by the model of Cousens (1985) at Allstar cultivar. YL=(1.4d)/[1+(1.4d)/23.22], where YL= estimated yield loss and d=weed density.

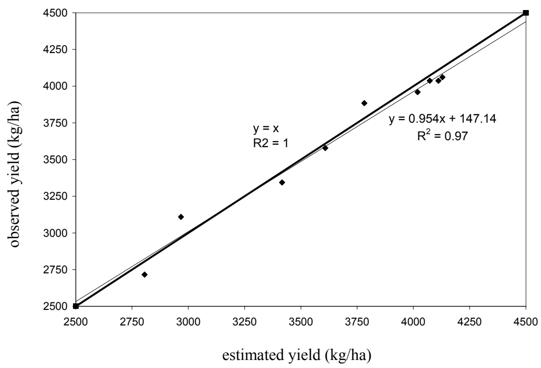

Fig. 5. Comparison of observed yield vs estimated yield by the model of Cousens et al. (1987) at Azarghol cultivar. YL= 4171[1-3.89d/100(Exp(0.14*t)+(3.89d)/41.00)], where YL= estimated yield loss and d=weed density, and t= relative interference time of weeds.

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Sunflower protection from negative effects of 2,4-D

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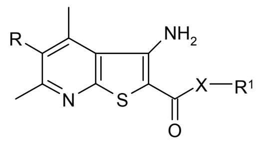
ABSTRACT
Twelve novel chemical derivatives of thieno[2,3-*b*]pyridines were synthesized and tested, aiming to find new compounds to protect/increase crop selectivity from herbicide impacts. Among these compounds, those with positive effect in reducing the toxicity of 2,4-D on sunflower are herein described.

Key words: 2,4-D – antidote – sunflower – thieno[2,3-*b*]pyridines.

INTRODUCTION

The use of herbicides to control weeds faces the problem of protecting some crops with low selectivity/ tolerance to some of them. Occasionally some compounds are very aggressive to some particular crops, and therefore, could occur a risk of damaging neighboring highly sensitive crops (Pitina et al., 1986; Strelkov et al., 1997). In addition, inaccuracy and mistakes of operators are possible while applying herbicides. Up to date, some approaches to protect crops from damage from herbicides have been developed, including selection of cultivars, agricultural methods, application of sorbing materials, etc. (Pitina et al., 1986, 1994). One promising approach includes a search for and application of chemical antidotes. Earlier, the possibility to use some pharmaceuticals and plant growth regulators to protect sunflower plants from 2,4-D during their growing season was shown by us (Strelkov et al., 1995a,b, 1997).

The objective of this work was to continue a search for new effective substances to protect crops from herbicides similar to 2,4-D. For this purpose, a number of novel chemical compounds have been synthesized by us, which belong to the derivatives of thieno[2,3-*b*]pyridines and have the following common formula:



where R = H, CI; X = O, NH, N; R^1= substituted phenyl, alkyl.

The compounds having this type of structure are known as biologically active compounds with a broad spectrum of activity (Litvinov, 1989); therefore, it seemed very useful to study their plant growth-regulating and antidote activity on sunflower seedling and adult plants.

MATERIALS AND METHODS

Germinating sunflower seeds with the embryo root of 2-4 mm in length were placed in the 2,4-dichlorophenoxiacetic acid (2,4-D) solution at the concentration of 10^{-3} % for one hour to inhibit the hypocotyls growth by 40-60%. After the herbicide action, the seeds were rinsed with water and put into solutions of the compounds tested for their antidote (safener) activity at the concentrations 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} % (herbicide + antidote treatment). An hour later, the seeds were washed with water and laid out on the filter paper bands (10 x 75 cm in size), 20 seeds per band. The bands were rolled up and placed into the beakers containing 50 ml of water. The further seed germination happened in the thermostat at 28°C for three days. The solution and rinsing water temperature was 28°C. The seeds from the "herbicide" treatment (comparison standard) were incubated in the solution of 2,4-D at the concentration of 10^{-3} % for one hour and then in water for the following one hour. The seeds from the control treatment were soaked in water for 2 hours.

The experiment included three replications; 20 seeds per replication were used. A protective (antidote) effect was determined by comparing an increase in hypocotyls and root lengths in the herbicide + antidote treatment with the same values in the herbicide treatment (standard).

The antidote field activity was evaluated in the ARRIBPP experimental field located in the central zone of the Krasnodar Region with moderate continental climate conditions. The soil in the field is a super-deep low-humic leached chernozem. The tests were conducted using the following methods: the sunflower plants of the cultivar Flagman, which were at the 10-16 leaf stage, were sprayed with butyl ether of 2,4-dichlorophenoxiacetic acid at the rate of 18 g/ha and, 5 days later, the tested antidote solution was applied at the rate of 200 g/ha by using the working fluid at the rate of 500 l/ha.

The experiment included the following treatments:

- Control – untreated plants;
- Herbicide (standard) – plants treated with herbicide;
- Herbicide + antidote – plants treated with herbicide and antidote.

The experiments were conducted in the 2.8 m^2 plots, with five replications. The plants were harvested with a Xere – 125 combine at the time of full seed ripeness.

An antidote effect was determined as a percentage by calculating the absolute yield gain value against the herbicide standard according to the formula:

$$A_x = \frac{A - E}{E} \text{ x } 100,$$

A_x – antidote effect, %;

A – yield in the herbicide + antidote treatment;

E – yield in the herbicide (standard) treatment.

The data obtained were statistically processed using Student's t-test at the probability level P = 0.95.

RESULTS

Twelve novel compounds derivatives of thieno[2,3-*b*]pyridines were synthesized using conventional methods (Shestopalov et al., 1988). Under the laboratory experiment conditions it was determined that the synthesized compounds had no growth-regulating effect on sunflower seedlings. At the same time, some compounds having an antidote effect were identified (Table 1).

The compounds deploying the most activity during the laboratory experiment were tested under the conditions of a field small-plot experiment. The results are given in Table 2.

Table 1. Antidote activity of the derivatives of thieno[2,3-*b*]pyridines having a common formula: against 2,4-D in sunflower seedlings (numerator:hypocotyl, denominator:root)

No.	R	X	R[1]	Control A[1]	Standard A	Treatment: Herbicide + antidote at the below concentrations, %							
						10^{-2}		10^{-3}		10^{-4}		10^{-5}	
						A	B	A	B	A	B	A	B
1	H	NH	2-bromphenyl	65	40	45	113	48	120*	46	115	40	100
				110	44	46	105	56	127*	57	130*	36	82
2	CI	NH	2- bromphenyl	65	40	47	118*	44	110	36	90	40	100
				110	44	57	130*	50	114	38	86	41	93
3	H	NH	3-fluorophenyl	77	36	43	119*	39	100	28	78	36	100
				115	32	47	147*	36	122*	27	84	32	100
4	CI	NH	3- fluorophenyl	74	51	59	116*	57	112	59	116*	55	108
				143	69	73	106	83	120*	81	117*	72	104
5	H	N	dipropyl	70	46	51	111	46	100	46	100	43	93
				125	61	69	113	63	103	61	100	53	87
6	CI	N	dipropyl	70	46	50	109	47	102	49	107	50	109
				125	61	67	110	55	90	61	100	57	93
7	H	NH	2,5-dimethoxy-	73	40	44	110	49	123*	49	123*	44	110
			4- chlorophenyl	116	42	56	133*	54	129*	68	162*	60	143*
8	CI	NH	2,5- dimethoxy-	73	40	41	103	34	85	40	97	46	115
			4- chlorophenyl	125	61	68	111	67	110	59	100	57	93
9	H	O	benzyl	76	37	49	132*	42	114	42	114	44	119
				120	47	51	109	51	109	38	81	38	81
10	H	O	allyl	76	37	52	141*	44	119	50	135*	49	132*
				120	47	42	89	44	94	42	89	44	94
11	CI	O	benzyl	77	44	56	127*11	54	123*	51	116*	53	120*
				91	44	63	43*	54	123*	55	125*	60	136*
12	CI	O	allyl	77	44	54	123*	45	102	43	98	45	102
				91	44	46	105	47	107	41	93	43	98

[1] A – average hypocotyl length, mm; B – increase in hypocotyl length compared to the standard, %.; * Reliable differences.

Table 2. Antidote activity of the derivatives of thieno[2,3-*b*]pyridines applied at the rate of 200 g/ha against 2,4-D in sunflower plants (field experiment)

Compound (Number given in the Table 1)	Treatment			
	Control (untreated)	Standard (herbicide)	Herbicide + antidote	
	Seed yield, g/ha			Gain against standard, %
2	2.83	0.89	1.43	161*
3	2.83	0.89	1.16	130*
7	2.04	0.75	1.11	148*
9	3.30	1.07	1.20	124
10	3.30	1.07	1.23	126*
11	3.19	0.94	0.99	105

* Reliable differences

DISCUSSION

As a result of screening novel compounds for their antidote activity against 2,4-D in sunflower, it was determined that a clearly marked and statistically reliable protective effect was produced by the derivatives of thieno[2,3-*b*]pyridines number 2, 3, 7, and 10. They contributed to the negative effect reduction of 2,4-D and increase in yield compared to the standard by 61, 30, 48, and 26 %, respectively (Table 2).

Under the laboratory experiment conditions, it was determined that the tested compounds did not have any growth-stimulating activity in sunflower seedlings; therefore, reduction in the phytotoxicity of 2,4-D could not be caused by its influence. It may be supposed that the herbicide action leveling was caused by one of the mechanisms described in the overview of Pitina et al. (1986).

The results obtained should be considered as the development of previous work and will expand the spectrum of protective means reducing negative effects of herbicides containing 2,4-D as part of their compositions. This is especially important in view of high sensitivity of sunflower to the herbicides of the 2,4-D group and insufficient research done in this field.

The results of the primary screening of novel antidotes (safeners) belonging to a series of thieno[2,3-*b*]pyridines led us to expect that, after adjusting their rates of application and spraying terms, they could be used to reduce negative impacts on 2,4-D on sunflower crops.

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Análisis del crecimiento de genotipos de girasol resistentes y susceptibles a herbicidas imidazolinonas

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RESUMEN
En la región central de los Estados Unidos de América especies autóctonas de *Helianthus* se comportan como malas hierbas en el cultivo de la soja. En 1996 se descubrió que dichos biotipos de girasol común son resistentes a herbicidas imidazolinonas, lo que dificultaba su control como mala hierba. Sin embargo, lo anterior abrió la posibilidad de transferir esa resistencia al girasol cultivado. A partir del cruzamiento de líneas resistentes a herbicidas imidazolinonas con los parentales del híbrido BRS 191, se obtuvo un genotipo resistente. Dicho genotipo se utilizó en el presente trabajo para realizar la comparación fenotípica con el híbrido BRS 191 susceptible, por medio del análisis de crecimiento. Los parámetros evaluados fueron: materia seca total, área foliar, materia seca de las hojas, materia seca de las raíces, materia seca del tallo, materia seca del capítulo, altura de las plantas, diámetro del capítulo, peso de mil aquenios, productividad, contenido de aceite, así como los índices de crecimiento relativo, asimilación líquida y área foliar. No hubo diferencia significativa entre ninguno de los parámetros evaluados, demostrando así que la incorporación del gen de resistencia a herbicidas imidazolinonas en los parentales del híbrido BRS 191, ha resultado en un genotipo con patrón de crecimiento similar al del BRS 191 susceptible. Este resultado abre la posibilidad de obtención de cultivares resistentes, que pueden ser importantes en el control de malas hierbas que afectan al cultivo del girasol.

Palabras-clave: *Helianthus annuus* - índice de crecimiento - resistencia genética.

Growth analysis of sunflower cultivars resistant and susceptible to imidazolinone herbicides

ABSTRACT
In USA Midwest, common sunflower is one of the main weeds of soybean. In 1996, a biotype of the common sunflower resistant to imidazolinones herbicides has caused much concern in the management of this weed. However, it also opened up the possibility of transferring this characteristic of resistance to the susceptible profitable cultivated sunflower. Starting from the crossing of American lines resistant to these imidazolinones herbicides with the parents of the hybrid BRS 191, a resistant genotype was obtained. This genotype was used in this study for phenotypic comparison with a normal BRS hybrid, through a growth analysis. The parameters evaluated were: total dry weight, foliar area, dry weight of leaves, root dry weight, stems dry weight, head dry weight, plant height, head diameter, weight of 1,000 achenes, productivity, and oil content. The relative growth rate, liquid assimilation rate, and the foliar area ratio were also estimated. There was no statistically significant difference between any of the parameters evaluated, demonstrating that the incorporation of the gene for resistance to herbicides of the imidazolinones group to the progenitors of the hybrid BRS 191 resulted in a genotype with a growth pattern similar to the susceptible BRS 191 hybrid. This finding opens up the possibility of obtaining resistant cultivars, becoming a highly important tool in the control of sunflower crop weeds.

Key words: genetic resistance – growth rate – *Helianthus annuus* – sunflower.

INTRODUCCIÓN
El girasol (*Helianthus annuus*) es una especie nativa de los Estados Unidos de América y sus poblaciones espontáneas, denominadas autóctonas o salvajes, se comportan como malas hierbas de cultivos tales como la soja y el maíz. Con el desarrollo de herbicidas inhibidores de la enzima acetolactato sintase (ALS),

selectivos para la soja, el control del girasol salvaje se ha llevado a cabo con éxito, por herbicidas de dicho grupo (Baumgartner et al., 1999). Sin embargo, la presión de selección provocada por el uso continuado de esos herbicidas ha proporcionado el desarrollo de biotipos de girasol resistente a los inhibidores de la ALS. En la mayoría de los casos, la resistencia de malas hierbas provoca mayor dificultad en el manejo de las infestantes y aumento en los costos de control. No obstante, en el caso del girasol salvaje, fue una oportunidad que se abrió para transferir esa característica genética para las variedades e híbridos cultivados, como relatan Miller y Al-Khatib (2001).

En Brasil, todavía no existe cultivo comercial de girasol resistente a los inhibidores de la ALS. Sin embargo, ello sería deseable, pues las malas hierbas dicotiledóneas son mayoría en las áreas de explotación de esa oleaginosa (Brighenti et al., 2003). De esa manera, en el año 2001 se inició en Embrapa Soja la introducción del gen de resistencia a los herbicidas del grupo de las imidazolinonas en genotipos de girasol de su banco de germoplasma, obteniendo en la cosecha de primavera/verano del 2003 tres genotipos F_4R_2 del cruzamiento entre líneas estadounidenses resistentes a las imidazolinonas y líneas nacionales susceptibles.

El objetivo de este trabajo ha sido comparar los genotipos de girasol resistente y susceptible a los herbicidas del grupo de las imidazolinonas, desarrollados por la Embrapa Soja, por medio del análisis de crecimiento de las plantas y sus características derivadas.

MATERIALES Y MÉTODOS

El experimento se desarrolló en condiciones de campo, en la Embrapa Soja. Se comparó el híbrido de girasol BRS 191 con un genotipo híbrido con las mismas líneas parentales, pero que recibieron la incorporación de la resistencia a las imidazolinonas, a través del cruzamiento con líneas estadounidenses seleccionadas por Al-Khatib y Miller (2000). También se utilizó el diseño enteramente aleatorizado, con cinco repeticiones. La siembra fue realizada el 10/03/04 y las evaluaciones fueron realizadas en intervalos de 14 días después del surgimiento de las plantas (DAE), que ocurrió en el 16/03/04. En cada evaluación se ha medido la altura de diez plantas por tratamiento, seleccionándose tres plantas por parcela al azar, que fueron cosechadas enteras, inclusive con el sistema radicular. Las raíces fueron lavadas en agua corriente para la retirada del suelo e impurezas, siendo, posteriormente, separados los órganos, el tallo, las hojas, las raíces y, después del florecimiento, también los capítulos. Cada órgano de las plantas fue puesto en fundas de papel y llevados para secar en estufa de circulación forzada de aire a 70 ± 1ºC, hasta alcanzar el peso constante, y posteriormente, pesado en balanza de precisión. Antes del secado, todas las hojas fueron utilizadas en la determinación del área foliar por medio de medidor fotoeléctrico de mesa, marca LI-COR, modelo 3100.

Los resultados de la altura de las plantas, del área foliar, de la materia seca de los órganos y de la materia seca total fueron sometidos al análisis de variancia, utilizándose el test F, a 5% de probabilidad y al análisis de regresión. En la cosecha del girasol se hizo la medición del diámetro medio de los capítulos, peso de mil aquenios, productividad y contenido en aceite. Los resultados fueron sometidos al análisis de varianza por el test F, siendo las medias comparadas por el test de Tukey, a 5% de probabilidad.

RESULTADOS Y DISCUSIÓN

Las curvas de acumulación de la materia seca total (MSt) de los genotipos fueron similares, prácticamente se solapan hasta los 47 días después de la aparición (DAE), y esa similitud se mantuvo hasta los 98 DAE (Fig. 1). La mayor acumulación de MSt ocurrió a los 86 días para el genotipo resistente y a los 87 días para el genotipo susceptible, con valores de 205, 73 y 196,52 g.planta^{-1}, respectivamente.

Como no hubo diferencia significativa en la MSt, la tasa de crecimiento relativo (Rw) también fue semejante para los dos genotipos, observándose gran ganancia de crecimiento hasta alrededor de los 30 DAE. Eso es normal en el girasol, pues ese periodo coincide con la fase vegetativa de este cultivo, que es aquella donde ocurre la formación y alargamiento de las hojas, iniciando con la germinación y terminando con la formación del pimpollo floral (Schneiter y Miller, 1981), siendo más expresiva en cultivares precoces, como es el caso del BRS 191.

Según Oliveira y Vieira (2000), el girasol BRS 191 inicia el florecimiento aproximadamente a los 53 DAE, cuando prácticamente cesa la formación y alongamiento de las hojas, alcanzando, por lo tanto, la máxima área foliar (Af). En este experimento, ambos los genotipos, resistente y susceptible, alcanzaron ese punto a los 53 DAE, con valores de 60,69 y 60,61 dm^2 planta^{-1}, respectivamente, mostrando diferencia mínima, no significativa, que se repitió en todas las evaluaciones.

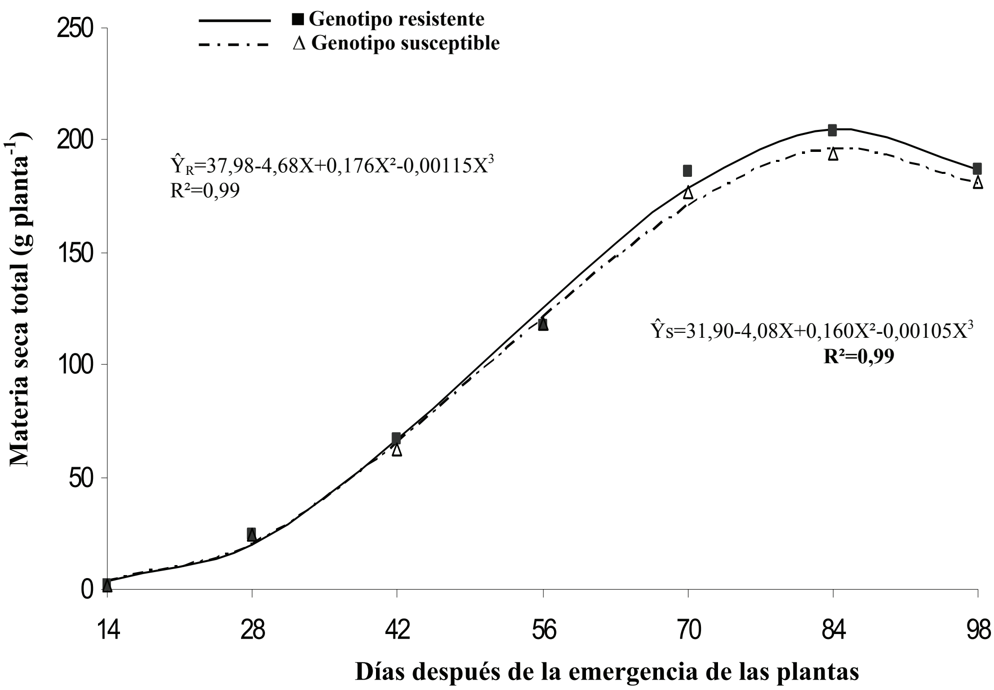

Fig. 1. Materia seca total de plantas de girasol de genotipos resistentes y susceptibles a las imidazolinonas.

La tasa asimilatoria líquida (TAL) es el parámetro que mide la producción de materia seca por unidad de área foliar, y tiene por objetivo analizar la eficiencia fotosintética de la planta. Ha habido una pequeña diferencia, no significativa, en esa tasa al inicio del desarrollo de las plantas, con mayor TAL de genotipo resistente con relación al susceptible, lo que no sucedió más a partir de los 28 DAE. Lo mismo observaron Brighenti et al. (2001), comparando biotipos resistentes y susceptibles de *Euphorbia heterophylla* a inhibidores de la ALS. Resultado inverso se obtuvo por Christoffoleti (2001) estudiando biotipos de *Bidens pilosa*, donde el susceptible obtuvo una TAL inicial superior al resistente. Sin embargo, en los dos trabajos los valores se aproximaron en las evaluaciones posteriores en las que el desarrollo de las plantas era mayor, asemejándose a lo observado en los genotipos de girasol.

Debido a los similares resultados del área foliar y de la MSt, la relación entre estos parámetros, representada por la razón del área foliar (Fa), tampoco ha resultado significativa la comparación de los genotipos. Los valores máximos de 1.82 y 1.80 $dm^2\ g^{-1}$ para el resistente y susceptible, respectivamente, se obtuvieron a los 29 DAE, disminuyendo en las evaluaciones posteriores con las curvas comportándose semejantemente a las de la TAL, pues a partir de la diferenciación floral ocurre la disminución progresiva de los fotoasimilados en dirección de las hojas (Vrânceanu, 1977). Después de la floración la senescencia y la caída de las hojas, contribuyendo para la reducción todavía mayor de la Fa. De esa forma no ha sido posible realizar la evaluación del área foliar y por consecuencia de la Fa en el proceso de la cosecha del experimento a los 98 DAE.

La materia seca de cada órgano del girasol, de las hojas (MSf), de las raíces (MSr), del tallo (MSc) y del capítulo (MScp), ha mostrado que no hubo diferencia significativa para ninguno de ellos. La acumulación de la materia seca de las hojas aumentó hasta los 66 DAE para los dos genotipos de girasol, resistente y susceptible, alcanzando valores máximos de 29.03 y 28.96 g $planta^{-1}$ respectivamente. El resultado demostró que el punto de máxima MSf ocurrió 14 días después de la máxima Af. Eso sucedió porque aún pasado el florecimiento pleno el girasol continúa manteniendo balance positivo de acúmulo de fotoasimilados en la hoja, por lo tanto, acumulando materia seca (Vrânceanu, 1977).

La materia seca acumulada de las raíces aumentó con la edad de las plantas y alcanzó los valores máximos de 21.68 y 20.86 g $planta^{-1}$, a los 81 DAE, para los genotipos resistente y susceptible respectivamente. Con relación a la materia seca del tallo, no hubo diferencia estadísticamente significativa. Los mayores acúmulos de materia seca fueron de 74.21 g $planta^{-1}$ para el genotipo resistente y de 69.29 g $planta^{-1}$ para el genotipo susceptible, ambos a los 79 DAE. Analizando la translocación de imazetapir en biotipos de girasol resistente y susceptible a las imidazolinonas, Al-Khatib et al. (1998) concluyeron que no existía diferencia entre los biotipos en la translocación del herbicida en el tallo hasta siete días después de su aplicación.

El peso de la materia seca de los capítulos, recolectado a partir de los 56 DAE, mostró la misma tendencia de acúmulo, con alto crecimiento de los 60 a los 84 DAE, pues es la fase en la que ocurre gran translocación de fotoasimilados para la formación y llenado de los aquenios. Esa fase ocurre, según Castiglioni et al. (1997), entre el final de florecimiento hasta la maduración fisiológica. La máxima MScp fue de 96.16 g planta^{-1} para el genotipo resistente y de 92.43 g planta^{-1} para el genotipo susceptible.

El crecimiento de las plantas de girasol tuvo mayor incremento en altura hasta los 28 DAE, resultado semejante al obtenido por Amabile et al. (2003) con la variedad Embrapa 122 también de ciclo precoz. El punto estimado de máximo crecimiento de los genotipos ocurrió a los 85 DAE, con altura de 175 cm del genotipo resistente y 179 cm del susceptible. No hubo diferencia significativa en ninguna época de evaluación.

Los similares resultados de los parámetros de crecimiento fueron además observados en los parámetros de rendimiento evaluados. No hubo diferencia significativa en el diámetro del capítulo, peso de mil aquenios, productividad y contenido de aceite entre los genotipos resistente e susceptible.

Por los resultados de este trabajo se concluye que la incorporación del gen de resistencia a los herbicidas de grupo químico de las imidazolinonas en los progenitores del híbrido BRS 191 resultó en un genotipo con semejante patrón de crecimiento al híbrido BRS 191 normal y susceptible, sin diferencias fenotípicas significativas. De esta forma se abre la posibilidad de obtención de cultivares, variedades o híbridos con la característica de resistencia a los herbicidas pertenecientes al grupo químico de las imidazolinonas, que puede transformarse en tecnología viable en el control das plantas dañinas en el cultivo del girasol en Brasil.

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Assessment of sunflower yield maps and discrimination of late-season weed patches by using field spectroradiometry and remote sensing: the case of *Ridolfia segetum* Moris

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ABSTRACT

Weed control strategies are commonly applied over the entire agricultural fields, although weeds are spatially distributed in patches. To reduce the consumption of herbicides applying them only where weed patches are present, it is necessary to develop accurate maps of weed patches. These weed maps can be obtained in late-season through high spatial resolution remote sensing and can be used for site-specific control next season. This is especially helpful taken into account that most weeds are stable in time and location. The main objective of this contribution is to describe the remote sensing requirements for predicting yield and mapping weeds, and to outline some results of our group in this research area by explaining the case study of *R. segetum* in sunflower. We have chosen this weed as it is one of the most widely distributed, hard to control and competitive broadleaf weeds in sunflower.

Key words: multitemporal aerial imagery – site-specific weed management – weed patch discrimination.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important crops in Andalusia (southern Spain) with over 240.000 ha grown annually (MAPA, 2006). It is normally grown under dry land conditions; sowing-time is February-March, and harvesting-time late July-mid August. One of the most frequent broadleaf weed species is the umbelliferous *Ridolfia segetum* Moris. It occurs in 25% of the sunflower surface in this region (Peña-Barragán et al., 2007) and two *R. segetum* plants per m^{-2} reduce crop yield by about 32% (Carranza-Cañadas et al., 1995). This weed is hard to control due to it not being controlled by pre-emergence and pre-plant incorporated herbicides used in sunflower, and, consequently post-emergence strategies such as tillage or hand weeding are commonly used, otherwise this weed obstructs the harvester due to it still having a partly green stem during the sunflower harvesting. Uncontrolled *R. segetum* plants also infest other crops included in the rotation, *e.g.* oilseed rape (*Brassica napus* L.) and medicinal-aromatic crops like anisette (*Pimpinela anisum* L.) generating serious contamination problems for oilseed rape oil and the anisette seeds for human consumption. Reduced and no-tillage production have increased in Spain in the last 10 years, and now account for 2.4 million of hectares of the annual crops (AESC/SV, 2005), many of them in Andalusia. So, *R. segetum* has become more troublesome since it cannot be reduced in abundance by repeated tillage or cultivation.

Patchy distribution of broadleaf weeds in sunflower fields is well documented (Jurado-Expósito et al., 2003). However, herbicide or other control strategies are not addressed to the infested zones, but they are usually broadcast over entire fields. The potential for overuse or application and corresponding eco-environmental problems is evident. One aspect of overcoming the possibility of minimizing the impact of herbicide on environmental quality is the development of Site-Specific Weed Management (SSWM). Timmermann et al. (2003) concluded that costs savings were 90% and 60% for broadleaf and grass weeds herbicides, respectively. A key component of SSWM is that accurate and appropriate weeds maps are required to take full advantage of site-specific herbicide applications. Mapping weed patches based on ground survey techniques on field scale is time consuming, expensive and unapproachable in field areas with difficult access. Remote sensing of weed canopies may be more efficient and suitable than field surveys and the majority of studies on discriminating weeds in cultivated systems have involved discrete broadband remote sensing (multispectral sensors) (Brown & Noble, 2005).

To detect and map weeds it is necessary for suitable differences to exist in spectral reflectance between weeds and crop or bare soil. Spectral reflectance differences can be enhanced by using Vegetation Indices, which are mathematical (ratio or linear) combinations between bands. Detection of late-season weed infestation has been demonstrated to have tremendous possibilities when spectral differences between crops and weeds prevail at a certain phenological stage (López-Granados et al., 2006). Taking into account that weed infestations are stable and persistent in location from year to year

(Jurado-Expósito et al., 2004), late-season weed detection maps can be used to design site-specific control methods in the coming 2 to 4 years. Thus, it is crucial to explore the variations in the spectral signatures of crop and weed, indicating suitable wavelengths for species discrimination and classification.

On the other hand, it is well known that crop yield varies spatially within the field since factors affecting crop growth such as soil properties, water availability, disease, weed and insect pressure, crop management practices, among others, vary spatially. Yield variability estimation during crop development could help farmers to make decisions (*e.g*. fertilization, irrigation, weed control) some time before harvest. Remote sensed imagery has been demonstrated to provide spatial and temporal georeferenced field information related to some field factors to predict yield estimation (Yang et al., 2006). As happens in weed mapping, one of the main challenges of remote imagery analysis in agriculture is to determine how variations in spectral information are related to differences in the crop phenological state, in order to obtain accurate yield maps long before the harvest, so that, crop management can be designed accordingly.

The main objective of this contribution is to describe the remote sensing requirements for predicting yield and mapping weeds and to outline some results of our group in this research area by explaining the case of *R. segetum* in sunflower. Our specific objectives were: 1) to determine the spectral signatures of bare soil, and different phenological stages of sunflower and *R. segetum*; 2) to select bands and Vegetation Indices for multispectral discrimination within-between phenological stages of sunflower and *R. segetum*, 3) to determine the ability to discriminate *R. segetum* patches on sunflower crops using aerial photographs, and 4) to assess the spatial relationship of sunflower yield to *R. segetum* weed presence.

MATERIALS AND METHODS

Study area: The study was conducted on two 40 ha sunflower fields located in Córdoba province (Andalusia, southern Spain), named Matabueyes and Santa Cruz, naturally infested by *R. segetum* and representative of infested areas in Andalusia. Sunflower crop Jalisco *cv*. was seeded at 4 kg ha^{-1} in rows 0.7 m apart in mid-March and harvested in mid-August. The field site was farmer-managed using shallow tillage production methods. Glyphosate was applied at pre-emergence at 0.7 l ha^{-1} for the control of annual weed seedlings. At this rate, this herbicide had no significant activity on *R. segetum*. Spectral reflectance signatures of bare soil, sunflower and *R. segetum* were measured from mid-May to mid-July according to the sunflower and weed phenological stages explained below.

Sunflower and R. segetum phenological stages: Sunflower and weed phenological stages were determined according to those adapted to our field conditions by Peña-Barragán et al. (2006).
A) Sunflower. 1) *mid-May, vegetative phase*: a) vegetative 5-10 leaves (SunV5-10), and b) reproductive head growing (SunHG); 2) *mid-June, reproductive phase*: c) reproductive head flowering (SunHF), and d) initial desiccation of lower leaves and reproductive head turning down (SunID); 3) *mid-July, senescent phase:* e) reproductive head partly desiccated and browning (SunRHPD); and f) plant completely desiccated and darkish/ black (SunPD).
B) R. segetum. 1) *mid-May, vegetative phase*: a) seedling < 5-10 cm, (RidSe); b) vegetative stage without floral stem (RidVe), and c) inflorescence (or umbella) still closed (RidInC); 2) *mid-June, flowering phase*: d) inflorescence yellowing, (RidInY); 3) *mid-July, senescent phase*: e) plant desiccated (RidPD).

Spectroradiometer data measurements and multispectral analysis: In mid-May, mid-June and mid-July, twenty hyperspectral measurements were collected for bare soil and each sunflower and *R. segetum* phenological stage using an ASD Handheld FieldSpec Spectroradiometer (Analytical Spectral Device, Inc., Boulder, USA) placed at 80-100 cm above each plant canopy or soil. Each measurement was georeferenced using the sub-meter differential GPS TRIMBLE PRO-XRS (Trimble, Sunnyvale, USA), to be located in the aerial images later. The spectral data were calibrated with a standard panel (Spectralon®) before each measurement. Measurements were made under sunny conditions between 12 and 14 h, and were collected between 400 and 900 nm (bandwidth of 1.5 nm).

Spectroradiometer data were averaged to represent the aerial imagery broad wavebands (blue, B: 400-500 nm; green, G: 500-600 nm; red, R: 600-700 nm; and near-infrared, NIR: 700-900 nm). The following vegetation indices (VI) were also calculated and analysed: Normalized Difference Vegetation Index NDVI = (NIR-R) / (NIR+R) (Rouse et al., 1973), Ratio Vegetation Index RVI = NIR / R (Jordan, 1969), R / B index (Everitt & Villarreal, 1987), VNVI = (NIR-G) / (NIR+G), and ANVI = (NIR-B) / (NIR+B). Multispectral data were subjected to analysis of variance, and means were separated at the 5% level of significance by LSD test using the SPSS software.

Aerial photographs: Conventional-colour (400–700 nm) and colour-infrared (500–900 nm) aerial photographs of the fields studied were taken in mid-May, mid-June and mid-July. Average flight height was 1525 m to obtain photographs at a scale of 1:10000. Selected photographs were digitised using the AGFA Horizon A3 scanner (635 dpi corresponding to pixels of 40 x 40 cm) and georeferenced (using 40 ground control points). ENVI 4.3 software was used to process images.

Image analysis: Two methods widely explained in Peña-Barragán et al. (2007) were applied to classify the images and to discriminate between the *R. segetum*-infested and the non-infested zones:
A) Class Separation Method: This was used in the four wavebands and the five Vegetation Indices previously described. Each image was classified by grouping the digital values according to the value ranges that characterized *R. segetum* training patches. Boundary digital values were established according to the statistical value obtained from 220 *R. segetum* training pixels, adding and reducing the standard deviation to the average.
B) Spectral Angle Mapper (SAM) Method: used for multispectral band images. It is based on an n-dimensional angle to match pixels to reference spectra made up of the digital signature of each training zone. The algorithm determines the similarity between two digital signatures by comparing their angles, treating them as vectors in a space with dimensionality equal to the number of bands. Smaller angles represent closer matches to the reference signature. The photo-interpreter has to specify the Maximum Angle Threshold in radians that set each land-use, so no pixels outside this threshold were classified.

During the field visits, 550 ground-truth pixels (infested and non-infested zones) were used to create the ground-truth images for every classification method. A numerical analysis called *Confusion Matrix* was then performed to quantify the accuracy of the coincidence between classification images and real patches for each classification method. The confusion matrix was used to obtain the overall accuracy (OA), which is the percentage obtained by dividing the pixels correctly classified among all ground-truth images. OA has been standardized in at least 85% for minimum accepted values (Thomlison et al., 1999).

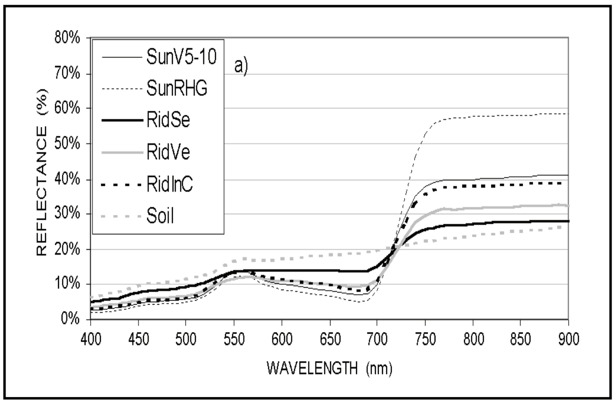
Sunflower Yield Data: The fields were harvested on 8th August using the farm´s MF34 combine harvester equipped with a differentially-corrected global positioning system (DGPS) receiver and a yield monitor with the Fieldstar® system (Massey Ferguson®, AGCO Corporation, Duluth, GA, USA). The yield data set used was of 2541 points, ranging from 0.30 to 2.30 t $ha^{-1.}$ A contour yield map of the complete field was then generated using the SURFER software (Golden software, Inc., Golden, Colorado, USA). Yield data set and yield map were grouped to six yield intervals (Very-Low=[0.30-0.60], Low=[0.61-0.95], Medium-Low=[0.96-1.30], Medium-High=[1.31-1.65], High=[1.66-2.00], and Very-High=[2.01-2.30] t ha^{-1}), to perform the statistical analysis. The four wavebands B, G, R, and NIR, and two vegetation indices (NDVI and NDYI) were considered to predict the yield map. Vegetation indices were calculated as follows: NDVI = (NIR - R) / (NIR + R); NDYI = (R - G) / (NIR + G).

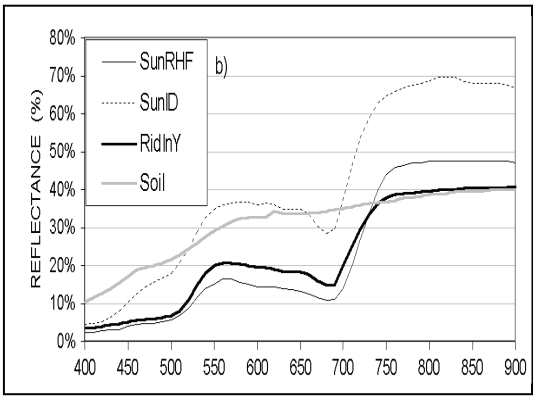
RESULTS AND DISCUSSION

Hyperspectral signatures of bare soil and phenological stages of sunflower and R. segetum: Reflectance curves of bare soil, and sunflower and *R. segetum* phenological stages corresponding to Mid-May (vegetative phase), mid- June (flowering phase) and mid-July (senescent phase) are indicated in Fig. 1. Phenological stages of crop and weed consistently affected the magnitude and amplitude of spectral reflectance values. Vegetative and flowering phases showed their characteristic higher reflectance in G (Green peak, 550 nm) and NIR (from 700 to 900 nm) parts of the spectrum. Senescent phase and bare soil presented their typical spectral signatures, *i.e.* reflectance values increased as wavelengths increased.

Multispectral analysis of bands and Vegetation indices: Results from analysis of variance and corresponding LSD test are shown in Table 1. B, NIR, NDVI, RVI, VNVI and ANVI values were statistically different between bare soil, and *R. segetum* and at least one of the sunflower phenological stages, suggesting that there is a potential for a successful discrimination between bare soil, sunflower and *R. segetum* using remote sensing.

Image classification method and weed map: OAs calculated throughout the confusion matrix for every classification method in mid-June (flowering phase) are listed in Table 2. Most classification methods studied, such as B, G, R, R/B, ANVI and SAM, discriminated *R. segetum* patches from bare soil and sunflower with OAs ≥ 85%. In particular, the best classifications of weed patches were obtained using SAM and R/B vegetation index, resulting in OAs of 95% and 98% in Matabueyes and Santa Cruz, respectively. Results obtained in mid-May and mid-July are not shown because they were generally poor (lower than 80%) and did not reach the commonly accepted requirement of at least an 85% classification of the overall accuracy. Therefore, it is not recommended to take any image in phenological stages corresponding to these dates to discriminate *R. segetum* patches.





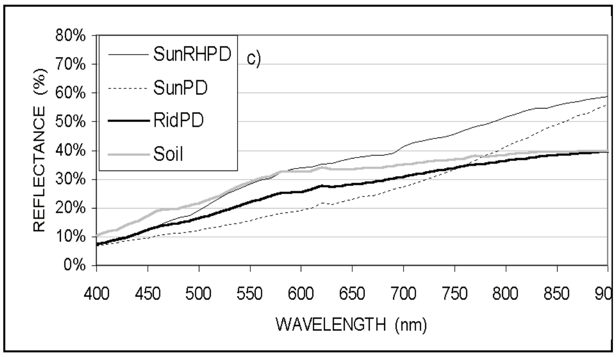


Fig. 1. Mean reflectance curves of bare soil and different phenological stages (*) of sunflower and *R. segetum* corresponding to three dates: a) mid-May (vegetative phase); b) mid-June (flowering phase), and c) mid-July (senescent phase).
(*See acronyms in Materials and Methods section).

Table 1. Mean reflectance values of bare soil, and sunflower and *R. segetum* phenological stages on three dates for multispectral bands and vegetation indices. (Best results are shaded in grey).

Dates	Phenological stages[2]	Mean values[1]								
		Multispectral Bands				Vegetation indices[2]				
		Blue	Green	Red	NIR	NDVI	RVI	RB	VNVI	ANVI
Mid-May	SunV5-10	0.04 b	0.10 ab	0.09 b	0.36 d	0.62 e	4.36 d	1.96 a	0.55 e	0.78 d
	SunRHG	0.03 a	0.09 a	0.07 a	0.50 e	0.76 f	7.61 e	1.93 a	0.69 f	0.87 e
	RidSe	0.07 d	0.13 c	0.14 d	0.26 a	0.30 b	1.86 a	1.93 a	0.34 b	0.56 b
	RidVe	0.05 c	0.10 ab	0.10 c	0.29 b	0.49 c	3.05 b	1.89 a	0.48 c	0.69 c
	RidInC	0.04 b	0.11 b	0.10 c	0.34 c	0.56 d	3.69 c	2.11 b	0.52 d	0.76 d
	Bare soil	0.09 e	0.15 d	0.18 e	0.23 a	0.13 a	1.30 a	2.01 ab	0.22 a	0.45 a
Mid-June	SunRHF	0.04 a	0.13 a	0.13 a	0.43 a	0.53 c	3.49 c	3.65bc	0.54 c	0.84 c
	SunID	0.10 a	0.31 b	0.33 b	0.64 b	0.36 b	2.40 b	3.14 b	0.37 b	0.74 b
	RidInO	0.05 b	0.16 a	0.18 a	0.37 c	0.36 b	2.16 b	3.86 c	0.39 b	0.76 b
	Bare soil	0.16 c	0.28 b	0.34 b	0.38 a	0.06 a	1.13 a	2.06 a	0.15 a	0.40 a
Mid-July	SunRHPD	0.12 b	0.27 c	0.37 c	0.51 b	0.17 c	1.40 c	3.01 c	0.31 c	0.61 c
	SunPD	0.10 a	0.16 a	0.23 a	0.41 a	0.29 d	1.80 d	2.42 b	0.45 d	0.62 c
	RidPD	0.12 b	0.22 b	0.28 b	0.36 a	0.13 b	1.30 b	2.40 b	0.26 b	0.51 b
	Bare soil	0.16 c	0.28 b	0.34 b	0.38 a	0.06 a	1.13 a	2.06 a	0.15 a	0.40 a

[1]Mean values followed by the same letter within a column for a single date do not differ significantly at the P [0.05%] according to LSD test.
[2]See Vegetation Indices and Phenological stages in Materials and Methods section.

Remote imagery vs. yield: Data presented are only from Matabueyes due to Santa Cruz's analysis still being in progress. The averaged wavebands and vegetation index data as affected by sunflower yield intervals and crop development stage are shown in Table 3. R waveband digital values and the NDVI index at the vegetative crop stage (mid-May) showed significant differences in all yield intervals. Furthermore, the NDVI mean value was negative for the three lowest yield intervals and positive for the three highest ones, and their values increased as the yield intervals increased. Mean values for the 6-sunflower yield interval map for NDVI index in mid-May are shown in Fig. 2a.

Table 2. Overall accuracy values (%) of every classification method in mid-June (Flowering phase). (Best results are shaded in grey).

Locations	Classification methods									
	SAM*	Blue	Green	Red	NIR*	NDVI§	RVI§	R/B§	VNVI§	ANVI§
Matabueyes	95	79	88	84	--	--*	--*	86	--*	--*
Santa Cruz	83	85	88	87	777	83	79	99	80	84

(*) SAM, Spectral Angle Mapper; NIR: Near-infrared band of Matabueyes field in mid-June could not be obtained due to technical problems and, thus, Vegetation Indices with NIR were not calculated .
(§) See Vegetation Indices in Materials and Methods section.

The NDYI index was only significantly different in the four highest yield intervals, and their mean values were positive and very similar for the three lowest yield intervals and negative for the three highest ones. In the flowering crop stage (mid-June), there were significant differences in B and G wavebands and in NDYI index (Table 3). On this date, NDYI mean values were negative in all yield intervals and inversely correlative with the increase in the yield intervals, ranging from -0.02 to -0.17. Higher NDYI values were found in mid-June than in mid-May due to greater differences between R and G values being obtained in mid-June. In mid-July (senescent stage), data are not shown because no significant differences were found between yield intervals and any bands or vegetation indices.

Considering the three dates, NDVI and NDYI corresponding to vegetative and flowering stages (mid-May and mid-June images) produced the best results, and their mean values correlatively increased as the yield increased, and decreased as the yield increased, respectively.

Table 3. ANOVA analysis of means for sunflower yield map, elevation, and bands and vegetation index data according to airborne imagery collected in mid-May and mid-June.

Yield Interval	Airborne image in mid-May						Airborne image in mid-June			
	Blue	Green	Red	NIR	NDYI	NDVI	Blue	Green	Red	NDYI
Very-Low	179 e	191 e	204 f	112 a	0.03 d	- 0.37 a	68 a	82 a	81 b	-0.02 f
Low	178 e	191 e	198 e	162 b	0.02 d	- 0.15 b	80 d	98 d	94 e	-0.04 e
Medium-Low	159 d	175 d	179 d	179 c	0.02 d	- 0.01 c	76 c	93 c	84 c	-0.08 d
Medium-High	142 c	157 c	155 c	180 cd	- 0.01 c	0.07 d	74 b	88 b	77 a	-0.10 c
High	129 a	135 b	130 b	181 d	- 0.03 b	0.14 e	96 e	102 e	89 d	-0.12 b
Very-High	136 b	123 a	116 a	206 e	- 0.08 a	0.33 f	107 f	105 f	89 d	-0.17 a

R. segetum pressure vs. sunflower yield intervals: The presence of *R. segetum* according to each yield interval and the weed influence on the reduction in the total yield are shown in Fig. 2b. This figure shows that weed presence diminished where yield increased. At the lowest yield intervals, R. segetum infested between 15 to 20 % of the total surface, but from medium-high yield to up upwards, the infestation was reduced from 14 to 3 %, suggesting that the zones within the high sunflower yield were sensitive to the absence of weed infestation.

CONCLUSIONS

Our results demonstrated that remote sensing images taken in mid-June can be a useful tool for both: 1) to estimate sunflower yield variability that can be used to generate a yield map for within-season identification of problematic or stressed areas for further site-specific management some time before harvest, and 2) to estimate late-season *R. segetum* patch maps that can be used in subsequent years for site-specific control strategies due to uncontrolled weeds being stable in location over the years in the field. The key question in predicting yield maps and mapping weed patches in crops is related to the time interval in which weed patches and crops show consistent and significant spectral differences. This paper concluded that aerial images taken in mid-June, which corresponds to the flowering phase of *R. segetum* and sunflower plants in our climate conditions, is the appropriate time to take aerial images for successfully completing the map of *R. segetum* patches and the expected sunflower yield.

a) b)

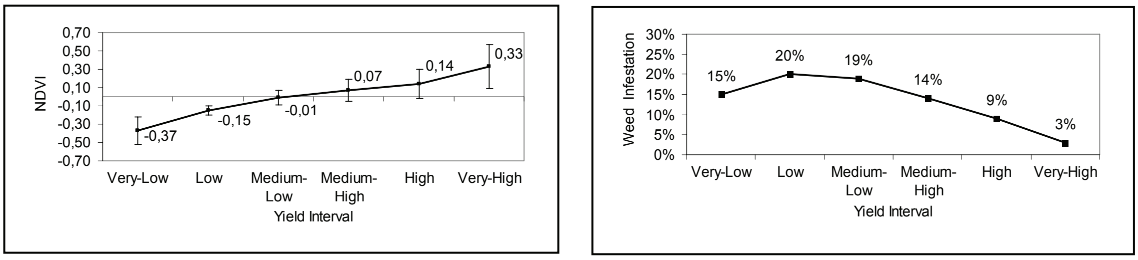


Fig. 2. a) Mean values of the 6-yield interval map for NDVI index in mid-May; b) Percentage of field surface infested by *R. segetum* according to 6-sunflower yield intervals.

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Control of *Cirsium* and *Xanthium* in sunflower hybrids resistant to the herbicide Express 50 SX

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ABSTRACT
Field research was carried out in four locations and during two years to study the selectivity and efficiency of the herbicide Express 50 SX applied in post-emergence to sunflower hybrids resistant to sulfonylurea herbicides.

Key words: dicots – herbicide – post-emergence – sulfonylurea – sunflower –weeds.

INTRODUCTION
Sunflower is one of the most important crops, strongly competed for by weeds from the first vegetation stages, especially in spring with low temperatures, which lead to slight growth of plants (Jonson, 1971). The sowing time is also of a special importance for early weed species, which could influence, at a high infestation, the yield level (Vannozzi et al., 1990).

On sunflower, several herbicides were first developed to be applied before and after sowing (trifluraline, linuron, metobromuron) for the control of annual weeds (Monotti, 1980). Afterward, attention was given to the association of herbicides such as trifluralin, alachloro, metolachloride, pendimethaline, linuron, prometrin, fluorochloridone, oxifluorfen (Lauretti, 1985; Tei et al., 1991; Millet et al., 1992). The research results have shown that a high efficiency in sunflower weed control is achieved by the association of chemical and mechanical treatments (Pintilie, 1986; Şarpe, 1987). In Romania (1970-1992), sunflower weed control was only partially solved because most dominant weeds (*Xanthium, Cirsium, Abutilon, Datura*) were not controlled by any herbicide. This problem was solved by the development by the Pioneer company, in collaboration with Cyanamid researchers (after 1990), of the first “genetically unmodified” sunflower hybrids “IR”, resistant to imidazolinone systemic herbicides, with effect in resistant dicots control: *Xanthium, Abutilon* and, partially *Cirsium*. This research performed world-wide after 2000 contributed to the development of the first “genetically unmodified” hybrids, resistant to tribenuron (Express 50 SX) of great importance under Romania conditions, due to the efficiency of Express 50 SX herbicide in post-emergence application, to control “problem” weeds: *Xanthium, Cirsium, Abutilon*.

The main aim of the research performed in Romania was to establish the optimum strategy to control mono- and dicots (including *Xanthium, Cirsium*) in sunflower crop with hybrids resistant to sulfonylurea (Express 50 SX).

MATERIALS AND METHODS
The experiments were performed during 2004-2005, at NARDI Fundulea, ARDS Lovrin, Oradea and Teleorman, with various weed infestations depending on the pedoclimatic conditions. The experiments were organized as randomized blocks, with plot area of 25 m^2, in four repetitions. Each plot was sown with 4 rows, with distance between rows of 70 cm. The cultivated hybrid was XF 4419, belonging to the Du Pont company. In the experiment plots, the herbicides mentioned in Table 1 were applied in post-emergence (sunflower: 4-6 leaves and 6-8 leaves stages), Also, the “split application” was employed (sunflower, 2-3 leaves and 6-8 leaves at re-infestation). For herbicide treatment, 250-400 l water/ha were used.

RESULTS AND DISCUSSION
The paper presents the results obtained during 2004-2005 at the research stations Lovrin, Teleorman, Oradea and NARDI Fundulea, placed under various climatic conditions, especially with a highly diversified infestation degree, weed spectrum and dominance. On average, the experiments presented strong infestations (80-95%) with annual and perennial mono- and dicots, with dicots prevalence (65%).

Table 1. Experimental details

Year	No.	Treatment	Rate a.i g/ha	Time of application	Content a.i g/l	Company
2004	1.	Untreated	-	-	-	
	2.	DPX.$_{75}$WG+Trend**	15 + 0.1%	Postem (4-6 lves)	75% tribenuron+Adj.	
	3.	DPX$_{75}$WG+Trend**	15+ 0.1%	Postem (6-8 lves)	75% tribenuron+Adj.	
	4.	DPX$_{75}$WG+Trend** + DPX$_{75}$WG+Trend	7.5+0.1%+ 7.5+0.1%	EPO (2-3 lves) +Reinf.(6-8 lves)	75% tribenuron+Adj.	Du Pont
	5.	DPX$_{75}$WG+Trend + Reset	15 + 0,1% + 37,5	Postem (4-6 lves)	75% tribenuron+Adj. + 50 g/l quizalofop P-etil	
	6.	Raft 400* (standard)	600	Postem (4-6 lves)	400 g/l oxidiargil	Bayer
2005	1.	Untreated	-	-	-	-
	2.	Raft 400 (standard)*	600	Postem (4-6 lves)	400g/l oxidiargil	Bayer
	3.	Express 50 SX**	15	Postem (4-6 lves)	50% tribenuron	Du Pont
	4.	Express 50 SX+ Trend**	15 + 0,1%	Postem (4-6 lves)	50% tribenuron + Adj.	Du Pont
	5.	Express 50 SX+Trend+ Fusilade s.	15 + 0,1% + 187	Postem (4-6 lves)	50% tribenuron + Adj.+ 125g/l fluazifop	Du Pont, Syngenta
	6.	Express 50 SX**	15	Postem (6-8 lves)	50 % tribenuron	Du Pont
	7.	Express 50 SX + Trend**	15 + 0,1%	Postem (6-8 lves)	50 % tribenuron + Adj.	Du Pont
	8.	Express 50 SX+ Trend+ Fusilade s.	15 + 0,1% + 187	Postem (6-8 lves)	50 % tribenuron + Adj.+ 125g/l fluazifop	Du Pont , Syngenta

*Graminicide herbicide pre-emergently applied
**Graminicide herbicide post-emergently applied

As dominance, the most representative ones were: *Cirsium, Xanthium, Sinapis, Raphanus, Chenopodium, Amaranthus, Hibiscus, Polygonum persicaria, Anthemis, Convolvulus* and only 35% annual mono-: *Echinochloa, Setaria.* In 2005, at Fundulea, with an infestation level of 90%, mono-species were predominant (65%): *Sorghum* (seed and rhizomes), *Echinochloa, Setaria*, while the dicots (35%) had a lower level of infestation: *Amaranthus, Chenopodium, Xanthium, Sinapis, Cirsium, Convolvulus.*

During two years of research, with enough rainfall after treatment, (30,2-128,8 mm in 20 DAT) at the application of herbicides DPX 75 (20 g/ha) and Express 50 XS (30 g/ha) associated with adjuvant Trend (0.1%) at two stages (4-6 and 6-8 leaves stages), phytotoxic symptoms were not recorded, as compared to standard treatment which showed leaves necrosis (averaged EWRS quotation 2.1 in 2004 and 2.3 in 2005), as we noticed in Table 2.

In 2005, at Fundulea (Fig. 3) in the variants treated by Express 50 SX alone or in association -Trend, Fusilade -in optimum stage (sunflower 4-6 leaves), the dicots control (including Cirsium, Xanthium) was higher (90-98% in 14-28 DAT) than 2004 due to lower dicots infestation (35%). In late application (sunflower 6-8 leaves) of the Express 50 SX herbicide, it obtained a lower effect in dicots control (85-93%, in 14-28 DAT) compared to the treatments applied in optimum stage.

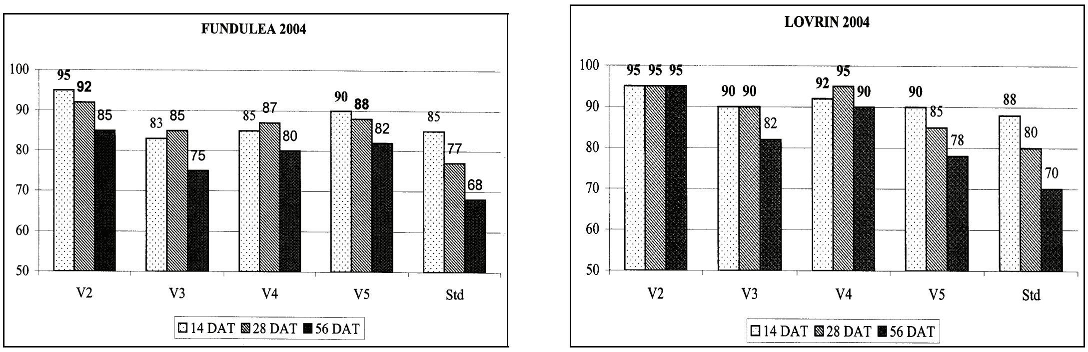
In 2004, at four stations (Fig. 1 and 2), at the application of the above mentioned herbicides to control dicots (especially the resistant ones: *Xanthium, Cirsium*), a superior efficiency (92-96%) was recorded as compared to standard variant (Raft 400) with control effect of 85-68% due to non control of resistant dicots (*Xanthium, Cirsium*). During the two experimentation years, the highest results in 14-28 DAT (92-96% -2004 and 96-97%, 2005), in dicots controlling (including the resistant ones: Xanthium, Cirsium) were recorded in the variants treated by DPX 75 WG (20 g/ha) or Express 50 SX (30 g/ha) + Adjuvant, applied in optimum stage for weeds and sunflower plants.

Table 2. The selectivity (EWRS quotation) of herbicides, postemergently applied, to control dicots in sunflower with hybrids resistant to Express 50 SX

Year	No	Variants	Rate a.i g/ha	Time of application	Selectivity (EWRS quotation) 2004				
					7 DAT	14 DAT	28 DAT	56 DAT	Mean
2004	V1	Untreated	-	-	1	1	1	1	1
	V2	DPX.$_{75}$WG+Trend**	15 + 0.1%	Postem. (Sunfl., 4-6 lves)	1	1	1	1	1
	V3	DPX$_{75}$WG+Trend**	15 + 0.1%	Postem. (Sunfl., 6-8 lves)	1	1	1	1	1
	V4	DPX$_{75}$WG+Trend** + DPX$_{75}$WG+Trend	7.5+ 0.1%+ 7.5 + 0.1%	EPO (2-3 lves) Reinf. (6-8 lves)	1	1	1	1	1
	V5	DPX$_{75}$WG+Trend + Reset	15 + 0.1%+ 37.5	Postem. (Sunfl., 4-6 lves)	1	1	1	1	1
	V6	Raft 400* (standard)	600	Postem. (Sunfl., 4-6 lves)	3	2^5	1^5	1	2^1
2005	V1	Untreated	-	-	1	1	1	1	1
	V2	Raft 400* (standard)	600	Postem. (Sunfl., 4-6 lves)	3^5	2^7	1^8	1	2^3
	V3	Express 50 SX**	15	Postem. (Sunfl., 4-6 lves)	1	1	1	1	1
	V4	Express 50 SX + Trend**	15 + 0.1%	Postem. (Sunfl., 4-6 lves)	1	1	1	1	1
	V5	Express 50 SXTrend + Fusilade	15+ 0.1%+187	Postem. (Sunfl., 4-6 lves)	1	1	1	1	1
	V6	Express 50 SX**	15	Postem. (Sunfl., 6-8 lves)	1	1	1	1	1
	V7	Express 50 SX + Trend**	15+ 0.1%	Postem. (Sunfl., 6-8 lves)	1	1	1	1	1
	V8	Express 50 SX + Trend + Fusilade	15+ 0.1+187	Postem. (Sunfl., 6-8 lves)	1	1	1	1	1

* Graminicide herbicide pre-emergently applied
** Graminicide herbicide post-emergently applied

Also, the best effect, 85-95% (2004) and 90-98% (2005) to control annual and perennial mono- and dicots was achieved in "tank mix" variant, using DPX 75, Express + Trend + graminicide (Reset or Fusilade), applied post-emergence, in optimum time (sunflower 4-6 leaves), being superior to standard treatment efficiency. The results show that the high efficiency to control mono- and dicots (especially the resistant ones) is directly correlated with rainfall before treatment, infestation degree, weed spectrum and prevalence as well as weed stage at treatment application.



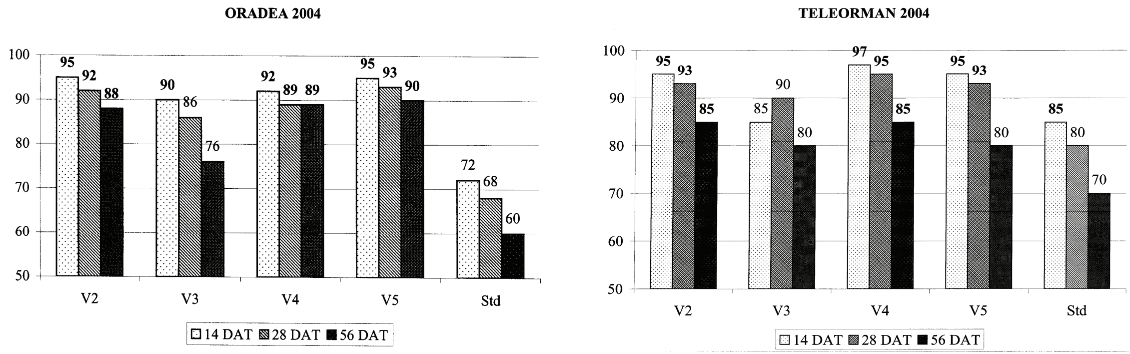
Infest. degree 85%
M/D 40/60
(Dp 35)
Weeds:

CIRAR	ANTAR
GALAP	PAPRH
XANST	ECHCG
AMARE	SETSP

Infest. degree 90%
(D/P 35/65)
Weeds:

SINAR	POLPE
CIRAR	CHEAL
CONAR	ECHCG
HIBTR	SETGL

Fig. 1. Efficiency (%) of herbicides, postemergently applied, to control dicots in sunflower with hybrids resistant to Express 50 SX in Fundulea and Lovrin in 2004.



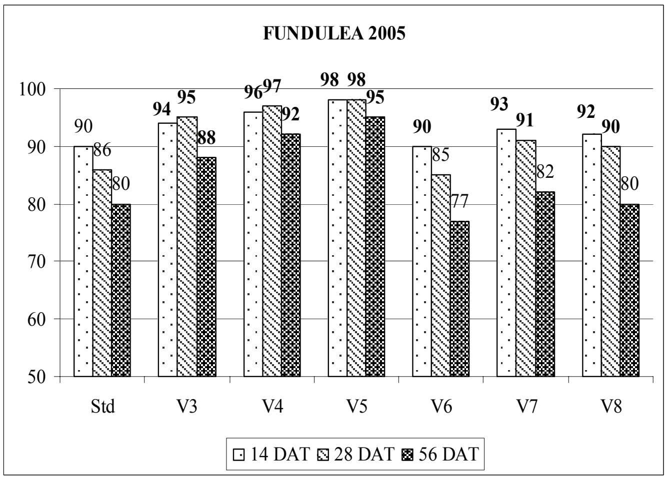
Infest degree -90%
M/D 20/80
Weeds:

XANST	POLSP
CIRAR	ANTAR
RAPRA	AMARE
CHEAL	ECHCG

Infest degree -90%
(M/D 35/65)
Weeds:

SOLNI	HIBTR
SINAR	AMARE
CIRAR	XANST
VIOAR	CONARCONAR
CHEAL	ECHCG

Fig. 2. Efficiency (%) of herbicides, postemergently applied, to control dicots in sunflower with hybrids resistant to Express 50 SX in Oradea and Teleorman in 2004.



Infest degree - 90%
M/D 65/35%
Weeds:
SORHA (s+r) AMARE
ECHCG CHEAL
SETGL XANST
SINAR
CIRAR
CONAR

Fig. 3. Efficiency (%) of herbicides, postemergently applied, to control dicots in sunflower with hybrids resistant to Express 50 SX

CONCLUSIONS

1. Herbicides DPX 75 and Express 50 SX, applied post-emergence (in both stages – 4-6 and 6-8 leaves) presented a very good selectivity (EWRS quotation =1) for sunflower "resistant" hybrids (XF 4419).
2. The selectivity degree recorded in variants treated with the above mentioned herbicides was superior (EWRS quotation=1) to classical treatment with herbicide based on oxydiargil (EWRS quotation=2^1-2^3).
3. Superior effect (over 90% in 14-28 DAT) was achieved in dicots control (including *Xanthium, Cirsium*) by post-emergence application of herbicides DPX 75, Express 50 SX + adj., at rate of 15 g. a.i./ha, sunflower 4-6 leaves stage.
4. The tested herbicide (in wet conditions) could be applied in association with herbicide based on fluazifop p-butil (in wet conditions), to control mono- and dicots, in "resistant" sunflower.
5. The application of herbicide based on tribenuron (single or with adjuvant), in late stage (sunflower 6-8 leaves) registers a diminution in its control of dicots (below 90%), especially on resistant species (*Xanthium, Chenopodium, Cirsium*), a re-growth taking place after treatment as compared to herbicides applied in optimum stage (sunflower 4-6 leaves; dicots 2-4 leaves).
6. The results obtained regarding the selectivity and efficiency to control dicots, at application of sulfonylurea systemic herbicides were superior to standard treatment – with contact herbicides (based on oxydiargil), which had no effect on "hard to control" species (*Xanthium, Cirsium*).
7. The establishment of an optimum strategy to control weeds in "resistant" sunflower was performed depending on climate conditions (before and after treatment), infestation level, weed prevalence and their development stage at the moment of application.

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Development of CLHA-Plus: a novel herbicide tolerance trait in sunflower conferring superior imidazolinone tolerance and ease of breeding

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ABSTRACT

A novel imidazolinone (IMI)-tolerance trait, CLHA-Plus, was developed through EMS mutagenesis and subsequent selection with imazapyr. The objective of this work was to determine the relative IMI tolerance level of this new mutation with respect to the current commercial IMISUN, by: (a) challenging genetic materials containing the new mutation and/or the old IMISUN with different doses of imazamox and imazapyr under a range of different environmental field conditions and (b) testing the *in vitro* acetohydroxyacid synthase (AHAS) activity of the CLHA-Plus and IMISUN hybrids at increasing levels of imidazolinone herbicides. Lines and hybrids homozygous for the CLHA-Plus mutation demonstrated better tolerance to imidazolinone herbicides than commercially available IMISUN sunflowers which are homozygous for the already known resistant gene (*Imr1*) and an uncharacterized modifier/enhancing factor (*Imr2*). Hybrids heterozygous for the combined mutations CLHA-Plus/IMISUN demonstrated similar field tolerance levels as well as similar AHAS enzyme IMI dose responses to hybrids homozygous for the novel CLHA-Plus mutation. Thus, a higher level of tolerance to imidazolinones can be achieved by allelic substitution of IMISUN by CLHA-Plus in only one of the parental lines of a CLEARFIELD® hybrid, which –in turn- permits a more rapid deployment of this new allele in the hybrid sunflower crop.

Key words: acetohydroxyacid synthase (AHAS) mutation – acetolactate synthase (ALS) mutation sulfonylurea – CLEARFIELD sulfonylurea – CLHA-Plus sulfonylurea – herbicide tolerance sulfonylurea – imidazolinone tolerance.

INTRODUCTION

The imidazolinone family of herbicides control weeds by inhibiting a key enzyme in the branched chain amino acid biosynthetic pathway, acetohydroxyacid synthase (AHAS) or acetolactate synthase (ALS) (Shaner et al., 1984; Tan et al., 2004). Imidazolinones, such as imazapic, imazethapyr, imazapyr and imazamox, are key herbicide components in the CLEARFIELD® production system, which provides effective and extended weed control when used in combination with elite non-GM, imidazolinone tolerant, seed varieties. The CLEARFIELD production system is used commercially in North America, Europe, South America, Asia, Australia and Africa in combination with the following crops: canola (oilseed rape), maize (corn), lentils, rice, wheat, and sunflowers (Pfenning et al. 2008).

The development of CLEARFIELD sunflowers started in 1996, when imidazolinone-tolerant (Pursuit®) wild sunflowers were discovered in a field in Kansas, USA. Seed of these plants were sent to the USDA in Fargo (North Dakota, USA) for subsequent crossing to cultivated sunflower lines (Al-Khatib et al., 1998)

The commercial imidazolinone tolerance trait, IMISUN, which arose from this original USDA introgression work, was commercially launched in the USA, Argentina and Turkey in 2004. From its initial launch up to the present, the IMISUN trait has seen growth in both the number of countries that have adopted this technology and in market share. Sunflower hybrid varieties are currently being commercialized under the CLEARFIELD trademark in 15 major sunflower growing countries in the European Union (EU), Eastern EU, North America (NA), and South America (SA).

The inheritance of IMISUN appears to be additively controlled by two genes, where one (*Imr1*) is a partially dominant gene and the other (*Imr2*) is a modifier or enhancer gene/factor (Miller and Al-Khatib, 2002; Bruniard and Miller, 2001). To produce IMISUN sunflower lines that express commercial tolerance levels to imidazolinone herbicides, both factors need to be homozygous in the final variety (*Imr1Imr1/Imr2Imr2*). Since there are no diagnostic methods available for detecting the presence of the

® CLEARFIELD and the unique CLEARFIELD symbol are registered trademarks of BASF modifier/enhancer factor *Imr2*, all breeding selections rely on the phenotypic evaluation of the plants that have been sprayed with the imidazolinone herbicide. This phenotypic selection process can be qualitative, depending on the segregation of the IMISUN genes, making the selection of homozygote lines often tedious and time consuming.

In an effort to develop a herbicide tolerance trait which does not require a modifier/enhancing factor, Nidera S.A. together with BASF initiated a seed mutagenesis program in sunflowers to discover new AHAS mutations that would simplify the breeding process. As a result, a new tolerance trait named CLHA-Plus was discovered (Sala et al., 2008). The objective of this work is twofold: (a) to determine the relative imidazolinone tolerance level of this new herbicide tolerance mutation with respect to the current commercial IMISUN and (b) to test the *in vitro* AHAS activity of the CLHA-Plus and IMISUN hybrids at increasing levels of herbicides.

MATERIALS AND METHODS

A sunflower line, BTK47, specifically selected for lack of an E-factor (*imr1 imr1 / imr2 imr2*), was subjected to EMS seed mutagenesis (Sala et al., 2008). An $M_{2:4}$ line which survived imazapyr field selection, was selected for subsequent crossing and enzyme activity studies. This line was named GM40.

Field Evaluation of the CLHA-Plus Trait

The CLHA-Plus mutant allele was introgressed into different maintainer, restorer and sterile inbred lines. Homozygous CLHA-Plus inbreds were crossed with either WT inbreds (containing no herbicide tolerance mutation), homozygous CLHA-Plus inbreds, or homozygous IMISUN inbreds to produce different F_1 mutant allele zygosity combinations (Table 1). These entries, along with several regionally adapted CLEARFIELD® IMISUN commercial variety checks, were field tested for imidazolinone tolerance at numerous locations in North America, South America and EU from 2005 to 2008 (Table 2).

Table 1. Entry list for herbicide tolerance field evaluations (2007)

Entry	Line Description	AHASL1 Allele Zygosity
1	GM40	CLHA-PLUS Homozygous
2	cmsGM40 x R733	CLHA-PLUS Homozygous
3	cmsBTK47 x R731	CLHA-PLUS Heterozygous
4	IA9 x R733	IMISUN / CLHA-PLUS Heterozygous
5	IA9 x RHA426	IMISUN Homozygous
6	B7imi (IMISUN1)	IMISUN Homozygous
7	cmsB7 x RHA426	IMISUN Heterozygous
8	B7	WT

Table 2. Location list for herbicide tolerance field evaluations (2005 - 2007)

Year	Country	Nearest Town Location, State or Province
2005	USA	Velva, North Dakota
2005/2006	Argentina (AR)	Venado Tuerto, Santa Fe
2006	USA	Velva, North Dakota
20062007	Argentina	Venado Tuerto, Santa Fe
20062007	Argentina	Balcarce, Buenos Aires
2007	Argentina	Laguna Blanca, Formosa
2007	USA	Velva, North Dakota
2007	USA	Hickson , North Dakota
2007	France (FR)	Angers
2007	France	Saintes
2007/2008	Argentina	Venado Tuerto, Santa Fe
2007/2008	Argentina	San Jeronimo, Santa Fe
2007/2008	Argentina	Balcarce, Buenos Aires

Table 3. Imidazolinone treatment list for herbicide tolerance field evaluations (2007)

Treatment Number	Herbicide Treatment	Herbicide Product Formulation
1	Untreated	
2	50 g ai/ha imazamox + 0.25% (v/v) NIS*	Beyond 120 g/l LC
3	100 g ai/ha imazamox + 0.25% (v/v) NIS*	Beyond 120 g/l LC
4	200 g ai/ha imazamox + 0.25% (v/v) NIS*	Beyond 120 g/l LC
5	160 g ai/ha imazapyr + 0.25% (v/v) NIS*	Arsenal 240 g ai/L
6	320 g ai/ha imazapyr + 0.25% (v/v) NIS*	Arsenal 240 g ai/L

*NIS = non-ionic surfactant = Induce 90SC (90%)

The entries at each location in 2007 and 2007/2008 were arranged in a randomized two factorial split plot design consisting of 3 replications for each treatment combination. Factor A was the herbicide treatment (Table 3), and factor B was the sunflower entry (Table 1). The plot size was 2 rows x 7 m and the seeding rate was consistent with local agronomic practices. The herbicide treatment was applied at the 2-4 leaf stage with a tractor mounted boom (20 gallons/acre or 200 litres/ha). Treatment 2 was only applied at 2 locations in France.

Crop injury (% phytotoxicity) ratings were evaluated at 6 - 10 days after treatment and at 16 - 21 days after treatment. Percent phytotoxicity was recorded as the average amount of plant damage in a given plot, where a rating of '0%' indicated no damage to plants relative to the untreated plot. A rating of 10% to 40% indicated increasing levels of chlorosis (where 40 would be complete yellowing of the leaves). A rating of 50% or higher indicated that the plants demonstrated complete yellowing as well as increasing levels of leaf necrosis. A rating of '100%' indicated complete necrosis (death) of the plants.

The emergence, days to flower, days to end of flower and maturity were also assessed for each plot at each location (data not shown). The data were subjected to an ANOVA analysis.

Enzyme Assay for AHAS Activity

Twelve greenhouse grown sunflower plants from each of the lines depicted in Table 4 were bulked and subjected to an AHAS enzyme activity assay (Singh et al., 1988). Each activity assay was repeated twice. Due to the large number of samples, the experiment was split into two sets (Table 4).

Table 4. Line descriptions and corresponding AHASL1 mutation allele zygosities

Set	Line Description	AHASL1 Allele Zygosity
1	cmsGM40 x R733	CLHA-PLUS Homozygous
1	IA9 x R733	IMISUN/CLHA-PLUS Heterozygous
1	IA9 x RHA426	IMISUN Homozygous
1	B7	WT
2	GM40	CLHA-PLUS Homozygous
2	cmsBTK47 x R731	CLHA-PLUS Heterozygous
2	B7imi (IMISUN1)	IMISUN Homozygous
2	cmsB7 x RHA426	IMISUN Heterozygous
2	B7	WT

Protein extracts from young, actively growing leaves from four week old plantlets were prepared and subjected to an AHAS inhibition assay (Singh et al., 1988). Assays were conducted in a 96-well format. Fifty µl of inhibitor was added to each well containing 50 µl of soluble protein extract to give final concentrations of 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50 and 100 µM imazamox or 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50 and 100 µM imazapyr. Zero herbicide controls were also included for each line. Reactions were processed as outlined by Singh et al. (1988). Absorbance was measured at 530 nm. AHAS activity, expressed as the mean of the absorbance values for each treatment, was presented as a percentage of the mean of the zero-herbicide controls.

RESULTS AND DISCUSSION

To assess HT genes for their relative tolerance level, two approaches were used. The first approach measured herbicide injury in the field under a range of environmental stringencies (locations and years in combination with different herbicide doses), and the second approach tested the target enzyme (*in vitro*) with increasing levels of herbicide.

In the field, the crop injury phenotype can be attributed to the interaction between genotype and environment (GxE). The genotypic factor in a herbicide tolerant (HT) plant is the sum of the HT gene(s) plus the remaining genetic background, and the interaction between the two. The environmental component (E) is a sum of abiotic (i.e. weather, soil) and biotic factors (i.e. insect, disease and weed pressure) coupled with the effect of the herbicide dose. An example of this environmental effect is seen in Fig. 1, where a variation in phytotoxicity of the same genotype grown in four different locations (Velva ND, Angers FR, Saintes FR, Formosa AR) at the same dose rate (200 g ai/ha imazamox) is observed. To better understand the environmental factor associated with this trait, we calculated the mean phytotoxicity index (PI) of the current commercial, regionally adapted, IMISUN checks at 6 – 10 days after herbicide treatment across many locations over 3 years. PI values for different hybrids carrying the CLHA-Plus mutation were plotted against the mean PI values of the IMISUN checks to evaluate the relative resistance level of the new mutation across a range of Es (Fig. 2). As can be seen in the x axis of Fig. 2, the combination of locations with herbicide doses produced a diverse array of Es, which ranged in PI mean values from 5.9 to 78 for the imazamox treatments (not shown); and 2 to 100 for the imazapyr treatments (Fig. 2). The y=x line represented the mean PI value for the IMISUN checks across all Es.

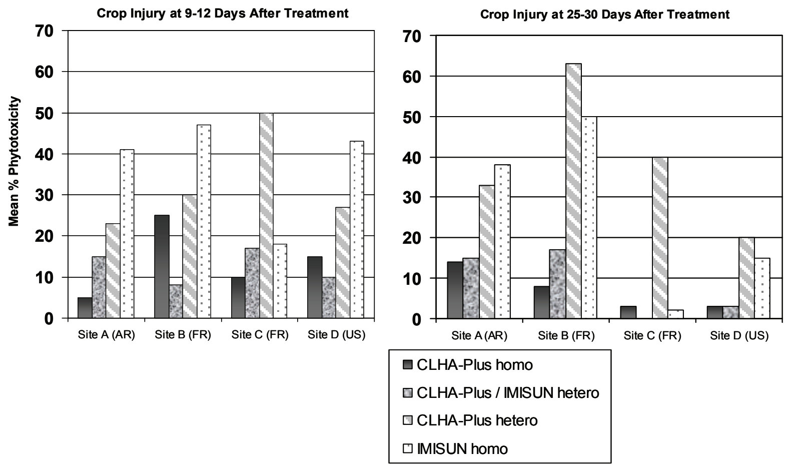

Fig. 1. Crop Injury (Mean % Phytotoxicity) at 200 g ai/ha Imazamox at 4 Field Locations in 2007 for 4 Different Types of Hybrids (see legend)

The results obtained following imazamox treatments are described in the following paragraph. The CLHA-Plus homozygous hybrids showed an increase in PI as the environmental component became more severe. However, the slope of the regression line (b= 0.149± 0.0667, P<0.0375) indicated that the level of crop injury, as a function of environmental stringency, increased at a lower rate than the IMISUN checks. Hybrids which were heterozygous for the double CLHA-Plus / IMISUN stack showed a similar response to environmental stringency (b=0.39±0.05, P<0.0001) as the homozygous CLHA-Plus hybrids. On the other hand, hybrids containing the CLHA-Plus mutation in a heterozygous state (CLHA-Plus/WT) demonstrated higher crop injury ratings than the IMISUN checks at lower levels of environmental stringency, as shown by the higher y-intercept value of the regression line (a=15.3±2.67). When the severity of the environmental component was increased, these CLHA-Plus heterozygous hybrids showed a better performance than the IMISUN checks, as was shown by the slope of its linear equation (b=0.45±0.062, P<0.0001). The same was observed in Fig. 2 when the same entries, in the same environments, were challenged with imazapyr (environmental stringencies for each genotype are summarized by the regressions in the Fig. 2 Legend).

To substantiate the herbicide tolerance effect observed in the field, the same herbicide tolerance gene combinations were subjected to AHAS enzyme inhibition studies. These studies were conducted on the bulk of 12 individuals from each entry in Table 1. The mean of two replications are represented in Fig. 3 for Set 1 (Table 1) and in Fig. 4 for Set 2 (Table 1). An untreated control sample was included to provide a baseline for 100% AHAS enzyme activity. The AHAS activity in the CLHA-Plus homozygous hybrid treated with 100 µM imazamox was 69% of the untreated control, and for the 100 µM imazapyr it was 64% of the untreated control (Fig. 3). The activity of the AHAS enzyme in the CLHA-Plus/IMISUN heterozygous hybrid was 59% and 60% for extracts treated with 100 µM imazamox and 100 µM imazapyr respectively (Fig. 3). The IMISUN homozygous hybrid line, current commercial CLEARFIELD® product, demonstrated AHAS activities of 36% of untreated control and 42% of untreated control at 100 µM imazamox and 100 µM imazapyr respectively (Fig. 3), which is lower than the activities of both the CLHA-Plus homozygous hybrid and the CLHA-Plus/IMISUN heterozygous hybrid.

In the second set of data, the IMISUN homozygous hybrid (current commercial CLEARFIELD product) performed similarly to the CLHA-Plus heterozygous hybrid (Fig. 4). More specifically, the IMISUN hybrid demonstrated 26% activity at 100 µM imazamox and the CLHA-Plus heterozygous hybrid had 30% activity at 100 µM imazamox. In contrast, the AHAS enzyme extract from the CLHA-Plus homozygous hybrid demonstrated the least amount of inhibition with increasing levels of imazamox, demonstrating activities of 63% and 60%, relative to the untreated control, at 50 µM and 100 µM imazamox respectively (Fig. 4). The WT line (B7) was genotypically identical in both experimental sets and demonstrated a variance of 6% activity at the 100 µM imazamox level between the two experiments (17% AHAS activity relative to the untreated control in Set 1 (Fig. 3) and 11% AHAS activity relative to the untreated control in Set 2 (Fig. 4).

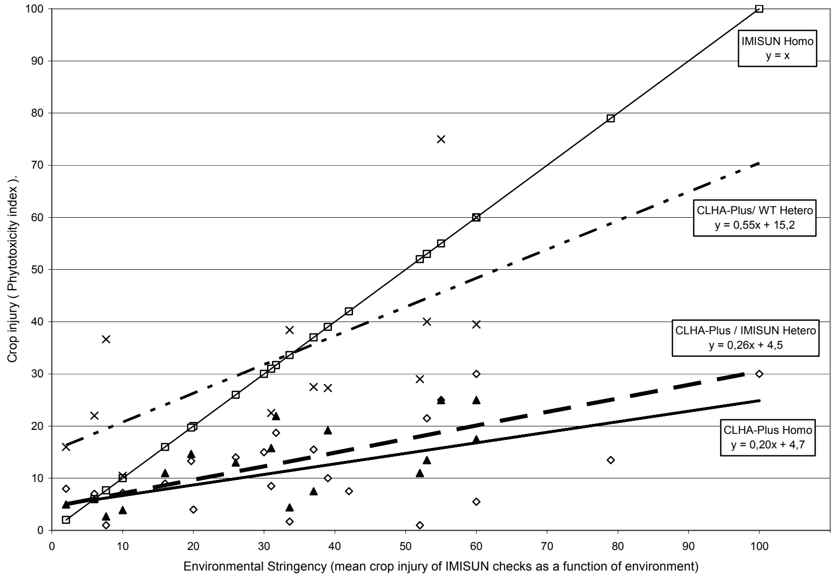

Fig. 2. Crop Injury of different types of sunflower hybrids carrying the CLHA-Plus mutation after imazapyr application ((CLHA-Plus homozygous: b= 0.20 ± 0.06, P<0.048 CLHA-Plus /IMISUN heterozygous: b: 0.26 ± 0.07, P<0.0019; CLHA-Plus /WT: b: 0.55±0.18, P< 0.0109)

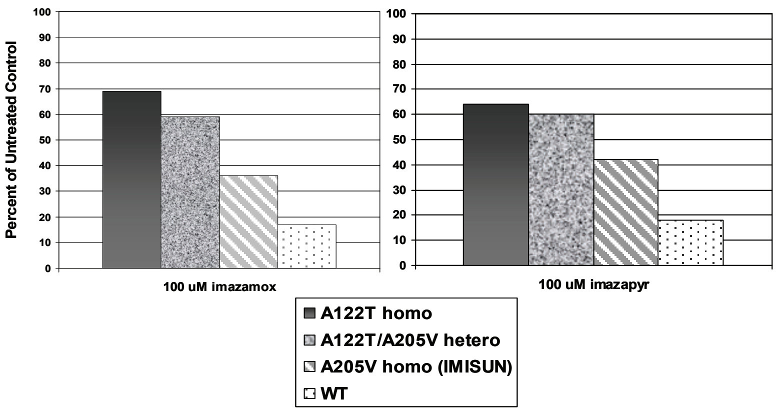

Fig. 3. AHAS Enzyme Activity (as Percent of untreated controls) of Four Sunflower Lines at 100 micromoles of Imazamox and 100 micromoles of Imazapyr

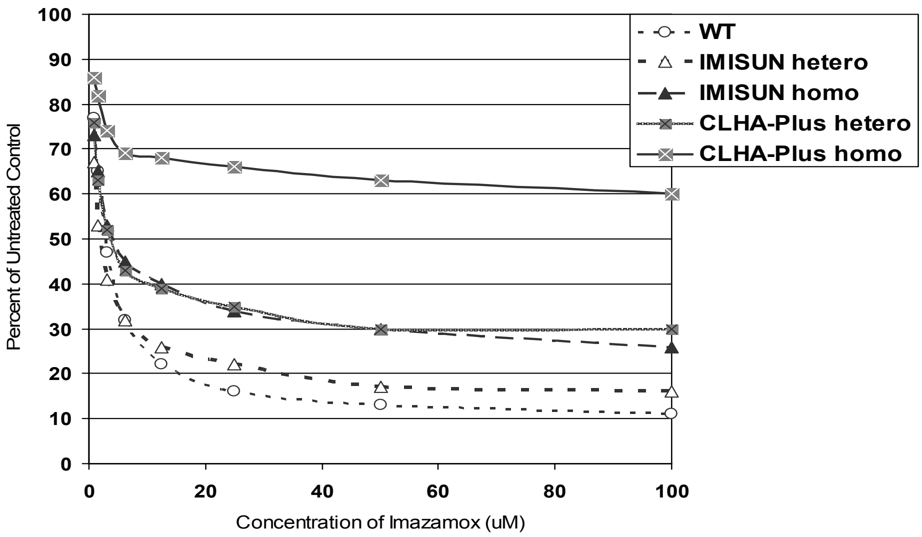

Fig. 4. AHAS Enzyme Activity (Percent of untreated controls) of Five Sunflower Lines with Increasing Levels of Imazamox

Based on field and AHAS enzyme activity data, it was determined that the novel CLHA-Plus mutation provides superior herbicide tolerance to imidazolinones versus the current IMISUN mutation. Commercial levels of herbicide resistance in IMISUN sunflowers require the combination of two genetic factors in a homozygous state due to the moderate level of resistance conferred by *Imr1*. In contrast, by using the CLHA-Plus mutation alone, the *Imr2* enhancer is no longer necessary to achieve commercial levels of tolerance. Most importantly, the results demonstrate that CLHA-Plus can be used either as a homozygous single gene HT trait or as a heterozygous stack together with the IMISUN HT trait, providing enhanced levels of tolerance, greater flexibility in weed control and facilitating the deployment of this new mutation in the CLEARFIELD® Production System.

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Selection of sunflower hybrids for Banja Luka area in Bosnia and Herzegovina

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ABSTRACT

Banja Luka area in Bosnia and Hezegovina does not have a regular sunflower production, although this area has favorable climate and soil conditions for profitable sunflower production. Based on three-year results of sunflower NS-hybrids developed at Novi Sad Institute of field and Vegetable Crops evaluated in micro experiments in the area of Banja Luka, we can establish the following conclusions: the highest average yield in a three-year period was accomplished by hybrids NS-H-411 and HN-H-45; the highest oil content in a three-year period was accomplished by hybrids Banaćanin and Kraišnik; the highest oil yield was accomplished by hybrids NS-H-411 and NS-H-45. In Banja Luka area of Bosnia and Herzegovina it is possible to achieve profitable production of sunflower on wider sowing areas, which could supply necessary quantity of oil as well as protein for livestock feeding.

Key words: Bosnia and Herzegovina – NS hybrids – oil content – oil yield – seed yield.

INTRODUCTION

Thanks to long-term selection work of Novi Sad Institute of Field and Vegetable Crops, today we have a great selection of high-yield sunflower hybrids for production in our agro-ecological conditions in Bosnia and Herzegovina. Our objective is to select high-yielding sunflower hybrids well adapted to agro-ecological areas in Bosnia and Herzegovina through long-term evaluation.

MATERIALS AND METHODS

We performed field evaluations in the period of 2004-2006 on a soil with acid reaction (pH=4.1) and low organic matter content (2.1%) at an altitude of 154 m. We evaluated 11 hybrids in 2004, 15 hybrids in 2005, and 13 hybrids in 2006. Evaluations were performed using randomized block design with four replications. The area of the plot was 14 m^2, with a distance between rows of 70 cm and a distance of plants in the row of 35 cm. Cultural practices were the standard for sunflower commercial production.

Climate parameters (Table 1) showed that the trials were conducted under optimal climate conditions. Average monthly temperatures in the three years included in the study were similar to long-term average temperatures (Table 1a). Precipitation during the vegetation period was greater than the long-term average (Table 1b).

Table 1. Meterological Parameters.

a) Average monthly temperatures (^oC).

Year	Month					
	April	May	June	July	August	September
2004	11.9	14.8	19.6	21.6	21.4	16.0
2005	11.8	16.3	19.4	22.0	19.4	17.0
2006	12.4	16.0	20.0	22.9	19.5	17.4
Average:	12.0	15.7	19.7	22.2	20.1	16.8
Long-term average:	10.9	16.1	19.3	21.4	21.1	16.7

b) Precipitation (mm).

Year	Month					
	April	May	June	July	August	September
2004	166.4	86.1	104.3	129.6	45.0	46.3
2005	80.5	79.2	135.6	129.7	124.9	79.7
2006	151.6	95.0	126.7	80.00	220.0	47.4
Average:	132.8	86.8	122.2	113.1	129.9	57.8
Long-term average:	80.3	95.0	113.7	87.1	71.6	90.6

RESULTS AND DISCUSSION

Long-term evaluation of NS-hybrids showed the great potential of sunflower production in Banja Luka area in Bosnia and Herzegovina. The high seed oil yield achieved demonstrated that it is possible to perform profitable production of sunflower in Bosnia and Herzergovina, which confirms previous results of Kondić (2004; 2005), Crnobarac et al. (2007), and Miklić et al. (2007).

The results showed that the hybrids with the highest yield potential were NS-H-411, NS-H-45, NS-H-43 and Somborac. Seed yield of these hybrids was between 3,775-4,402 kg/ha (Table 2). Oil content was found between 32.9% (Labud) and 46.81% (Baća). The hybrids with the highest seed oil content were Baća, Somborac, Banaćanin, and Kraišnik (Table 3). Oil yield ranged from 1,251 kg/ha (Labud) to 1,866 kg/ha (NS-H-411) (Table 3). The highest oil yield was recorded in the hybrids NS-H-111, NS-H-45, Kraišnik, NS-H-43, and Baćanin (Table 3).

Table 2. Seed yield of sunflower hybrids evaluated in Banja Luka area of Bosnia and Herzegovina.

Hybrid	Grain yield with 11% humidity (kg/ha)			Average
	2004	2005	2006	
NS-H-111	4,070	4,455	4,700	4,408
NS-H-45	4,190	4-312	3,700	4,401
Krajišnik	3,700	3,350	4,440	3,830
Bačvanin	3,290	3,895	3,760	3,658
Banaćanin	3,370	3,160	4,540	3,690
Velja	3,150	2,665	3,960	3,258
Perun	3,540	3,405	4,020	3,655
Olivko	3,100	3,200	3,510	3,270
Pobednik	4,050	3,510	2,970	3,510
Labud	3,660	3,945	-	3,802
NS-H-43	3,800	3,770	-	3,785
Šumadinac	-	3,300	3,200	3,250
Baća	-	3,230	3,110	3,170
Somborac	-	3,750	3,800	3,775
Sremac	-	3,605	3,400	3,502
Average:	3,656	3,570	3,778	
LSD 5%	553	391	244	
1%	735	519	326	

Table 3. Oil content (%) and oil yield (kg/ha) of sunflower hybrids evaluated in Banja Luka area of Bosnia and Herzegovina.

Hybrid	Oil content (%)			Average	
	2004	2005	2006	Oil content %	Oil yield kg/ha
NS-H-111	42.19	45.96	38.86	42.34	1,866
NS-H-45	40.57	46.33	39.72	42.21	1,858
Krajišnik	43.13	49.77	43.20	45.37	1,738
Bačvanin	45.05	45.23	40.46	43.58	1,590
Banaćanin	42.78	50.83	44.07	45.89	1,693
Velja	45.74	45.84	41.06	44.21	1,440
Perun	38.71	49.84	41.06	43.20	1,579
Olivko	40.57	45.44	45.04	43.68	1,428
Pobednik	38.86	49.44	45.89	44.73	1,570
Labud	36.13	29.70	-	32.91	1,251
NS-H-43	41.97	48.23	-	45.10	1,707
Šumadinac	-	46.60	41.81	44.20	1-436
Baća	-	49.11	44.51	46.81	1,484
Somborac	-	48.26	43.65	45.95	1,735
Sremac	-	45.61	42.20	43.90	1,537

The three-year evaluation of NS-hybrids of sunflower in the area of Banja Luka in Bosnia and Herzegovina led us to the following conclusions:

1. Climate and soil conditions are satisfactory for sunflower production in Bosnia and Herzegovina.
2. All examined hybrids gave average yield larger than 3 t/ha.
3. It is possible to increase yield potential by further evaluation of hybrids and through adoption of modern production techniques.

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Principal component analysis as a reflector of combining abilities

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ABSTRACT

In this study Principal Component Analysis was used to reveal two dimensional structures based on general (GCA) and specific combining abilities (SCA) in a sunflower crossing program. GCA and SCA of new sunflower inbred lines accompanied by genetic variance components were estimated using Line x Tester analysis method. Six new restorer lines were crossed with four CMS lines as tester in 2006. 24 combinations were planted in randomized block design with four replications in Khoy Agricultural and Natural Resources Research Station in 2007. All traits except head diameter and plant height were under control of both additive and dominant effects. Seed yield was mainly under control of dominant effects. For the growth period, and in other traits, additive effects had a major influence. Overdominant effects for growth period and seed yield, complete dominance for plant height and partial dominance for other traits were evident. Principal component analysis revealed a close positive association between seed yield, oil yield and seed number, whereas there were negative associations between these traits and 1000 seed weight. Principal components located entries based on their whole performance in the biplots provided. Restorer line R21 and CMS line CMS356 had a strong effect on their corresponding hybrids. Ordination biplots provided simplicity of selection based on agronomic means instead of SCA data. R23 x CMS78 was a superior early matured hybrid with higher oil content and yield. We could use PCA based on agronomic data alone instead of combining abilities for determining real performance of entries considering multivariate traits.

Key words: combining ability sulfonylurea – principal component analysis – sunflower sulfonylurea – variance components.

INTRODUCTION

Sunflower is one of the most important oilseed crops of Iran but its planting area has been very variable and recently decreased to 30000ha. Breeding programs in seed and plant investigation institutes are carried out for producing new hybrids to replace open pollinated (OP) varieties. Iranian Hybrids have not been very successful to date but a new generation of F1 hybrids is now being produced. A basic research program in this field has been focused on producing parental lines and many CMS and restorers have been produced. In these programs, estimation of combining ability of lines to identify superior parents for hybridization is essential. Besides an estimation of combining abilities and identifying the type of gene action governing yield-related traits, this study was made to establish a two dimensional structure between agronomic performance and combining abilities. Predominant of dominance gene action was reported for plant height, head diameter, oil content, 100 seed weight and seed and oil yield (Gangappa et al., 1997). However, additive gene action for these traits has also been reported (Singh et al., 1989) Estimates of GCA and SCA (Bedov, 1985) indicated that additive effects were more important for oil content. Additive gene action has the greatest influence on flowering (Alvarez et al., 1992). A significant relationship between morphological differences between inbred lines and SCA effects was found for oil yield and seed number per plant (Luczkiewicz and Kaczamarek, 2004). These relationships could be used to evaluate lines directly based on agronomic values instead of on combining abilities. Biometrical methods such as principle component analysis allow us to recognize structures between genotypes based on multivariate traits. Tersac and et al. (1993) used PCA based on SCA to show a structure of sunflower populations by country of origin. De la Vega et al. (2001) used PCA for revealing two dimensional structures among genotypes and environments based on their interactions. They reported the effectiveness of PCA for demonstrating genotype x environment interactions. In this study we have investigated the efficiency of PCA in screening genotypes due to GCA and SCA of multivariate traits.

MATERIALS AND METHODS

This experiment was conducted in Khoy-Iran Agricultural and Natural Resources Research Station in 2007. The station is located in 38º 32' north latitude and 44º 58' east altitudes. Six new restorer lines were crossed with four cytoplasmic male sterile (CMS) lines in line x tester fashion to generate 24 single cross hybrids in first year and twenty four F1 hybrids planted in randomized block design with four replications in 2007. Each experimental plot consisted of 3 rows of 4 m in length with 60cm spacing between rows and 25cm within rows. Fertilizers were applied at the rate of 100:70:90 NPK kg/ha. Field practices were followed according to the regional sunflower planting handbook (Ghaffari, 2006). Data of measured traits for hybrids subjected to Line x Tester analysis (Kemptorne, 1957) to estimate general combining ability (GCA), specific combining ability (SCA), effects and their respective variances were collected. Principal Component Analysis (PCA) was used to arrange the entries in two dimensional biplots (Kroonenberg, 1997) based on agronomic performance and combining abilities.

RESULTS AND DISCUSSION

There were significant differences between crosses for all measured traits but differences between CMS lines were greater than those between restorers (Table 1).All traits except head diameter and plant height were under control of both additive and dominant effects. Seed yield was mainly under the control of dominant effects. For growth period, the dominant effects were more important than additives and in other traits additive effects had a major influence. Over dominant effects for growth period and seed yield, complete dominance for plant height and partial dominance for other traits were evident (Table 2).

Table 1. Analysis of variance for agronomic traits

Sources	D.F	Growth Period	Plant Height	Head Diameter	1000 Seed weight	Seed Number /head	Seed yield	Oil Content	Oil Yield
Replication	3	44.9**	339.26	48.244**	314.2**	35628.6	1162745.4*	50.32**	182658.6
Crosses	23	32.4**	349.49**	5.445	154.7**	79052.1**	1155345.3**	18.12**	278046.6**
Lines	5	39.8**	550.8**	2.932	171.9**	34051.2	470879.6	33.48**	123211
Testers	3	68.2**	605.8*	5.653	610.33**	349693.9**	3627592.4**	58.64**	1043106**
Lines x Testers	15	22.8**	231.1	6.241	57.8*	39923.9*	889051.3**	4.89**	176646.7*
Error	69	6.6	160.9	5.373	28.7	20324.9	361540.3	7.039	86292.98
C.V.		2.42	6.86	12.47	7.42	21.73	19.29	5.87	20.80

* and ** significant at 0.05 and 0.01 level of probability respectively

Table 2. Variance components for agronomic traits

Variance	Growth Period	Plant Height	Head Diameter	1000 Seed weight	Seed Number /head	Seed yield	Oil Content	Oil Yield
Additive	3.13*	34.72*	-0.19	33.33**	15194.86*	116018.49+	4.12**	40651.16*
SE	1.24	12.40	0.12	9.94	5568.92	58699.98	1.03	16712.78
Dominance	4.03**	17.53	0.22	7.28*	4899.76*	131877.70**	0	22588.43*
SE	0.99	10.47	0.29	2.55	1764.04	38865.08	0.26	7787.033934
Dominance rate	1.61	1.00	0.66*	0.66*	0.80	1.51	0.00	1.05

* and ** significant at 0.05 and 0.01 level of probability respectively

Restorers R50, R21 and R23 had the highest GCA for seed yield but none of them was significant (Table 3). R21, R23 and R56 had significant positive GCA for 1000 seed weight, seed number and oil content, respectively. This indicates that these restorers seemed to possess increasing alleles with additive effects for the mentioned traits. R26 and R50 had significant negative GCA for growth period indicating presence of alleles with additive effects for early maturity. Therefore, R26 seemed to possess additive alleles for dwarfness. Single branch restorer RG50 distinguished itself as being a good line for using in crossing programs because of having the desired GCA for seed yield and growth period.

Testers CMS52 and CMS148 had the highest GCA for seed yield but only CMS52 had significant GCA in the desired (positive) direction (Table 4). CMS 356, CMS52 and CMS78 lines had significant positive GCA for 1000 seed weight, seed number and oil content, respectively. CMS356 seemed to have alleles with additive effects for increasing the growth period, while CMS52 had alleles for decreasing it. It would seem that CMS52, because of having the desired GCA for seed yield and growth period, could be used as a valuable A-line in crossing programs.

Table 3. GCA of restorer lines for agronomic traits

Restorer	Growth Period	Plant Height	Head Diameter	1000 Seed weight	Seed Number /head	Seed yield	Oil Content	Oil Yield
R19	0.07	-6.11	-0.32	-0.62	-50.91	-254.66	-1.28*	-158.63*
R21	-0.61	7.96*	0.58	-3.37	72.74*	114.51	-0.34	44.43
R23	2.51**	1.97	-0.43	5.41**	-28.18	82.43	1.14	82.07
R26	-1.55*	-7.72*	0.39	0.94	-31.40	-112.57	0.29	-41.77
R50	-1.49*	1.54	0.13	0.94	30.39	217.85	-1.82*	36.76
R56	1.07	2.37	-0.35	-3.31	7.38	-47.57	2.01**	39.20
SE	0.59	2.90	0.53	1.22	32.54	137.22	0.61	67.04

* and ** significant at 0.05 and 0.01 level of probability respectively

Table 4. GCA of CMS lines for agronomic traits

CMS	Growth Period	Plant Height	Head Diameter	1000 Seed weight	Seed Number /head	Seed yield	Oil Content	Oil Yield
CMS78	-0.70	2.19	-0.35	-2.41	21.10	23.96	1.31*	54.65
CMS52	-1.32*	3.47	0.69	-2.05	87.16**	318.68*	0.60	154.93*
CMS148	-0.45	-7.46**	-0.33	-3.09	67.96	211.18	0.35	97.01
CMS356	2.47**	1.81	-0.01	7.53**	-176.21	-553.82**	-2.26	-306.57**
SE	0.46	2.24	0.41	0.95	25.20	106.29	0.47	51.93

* and ** significant at 0.05 and 0.01 level of probability respectively

Hybrids R23 x CMS78 and R56 x CMS356 had high SCA for both seed yield and growth period (Table 5). These hybrids had seed yield of 4105 and 3022 kg/ha, respectively, and growth period of 107 days (Table 6). Crossing of R21, R26, and R50 with CMS52 and R21 with CMS148 resulted in higher seed yield (over 3500 kg/ha) with short growth period (107 days) and makes them high yielding early maturing hybrids for summer cropping.

Table 5. SCA of crosses for agronomic traits

Restorer	CMS	Growth Period	Plant Height	Head Diameter	1000 Seed weight	Seed Number /head	Seed yield	Oil Content	Oil Yield
R19	CMS78	-0.11	-1.51	1.18	1.13	-79.13	-308.12	-1.38	-172.54
R19	CMS52	-0.99	10.06*	-0.91	0.52	18.04	60.49	0.86	50.80
R19	CMS148	3.64**	1.31	0.53	1.94	59.81	406.32	-0.79	152.33
R19	CMS356	-2.53*	-9.89	-0.79	-3.56	1.28	-158.68	1.32	-31.98
R21	CMS78	-1.93	-3.96	-1.02	-7.25	-23.97	-403.96	1.46	-139.56
R21	CMS52	0.95	-2.46	1.92*	-1.60	149.51*	649.66*	-1.12	250.58*
R21	CMS148	0.57	2.09	-1.54	2.94	30.39	257.16	-0.46	109.74
R21	CMS356	0.41	4.30	0.64	5.94*	-155.94*	-502.85*	0.12	-222.15
R23	CMS78	-2.05*	-7.74	0.65	2.35	145.60*	881.47**	0.15	411.84**
R23	CMS52	-1.18	10.14*	-1.69	-0.88	-120.49*	-598.26*	0.74	-252.21
R23	CMS148	-2.05*	0.01	0.22	-0.22	-55.88	-222.43	0.11	-89.18
R23	CMS356	5.28**	-2.44	0.83	-1.22	30.76	-60.76	-1.00	-71.83
R26	CMS78	0.51	2.45	0.42	-0.56	-65.97	-350.21	0.80	-149.18
R26	CMS52	-0.11	2.32	-0.47	-0.54	44.58	235.07	1.07	148.78
R26	CMS148	0.26	-2.55	-0.35	-2.37	68.75	200.91	-0.35	71.64
R26	CMS356	-0.66	-2.25	0.41	3.50	-47.39	-85.76	-1.52	-72.63
R50	CMS78	2.45*	7.86	-1.07	1.94	-8.67	6.04	-0.48	-17.65
R50	CMS52	-0.43	-16.36**	0.54	-0.66	13.36	29.66	-0.40	3.84
R50	CMS148	-2.05*	-0.01	1.91*	-3.00	-37.44	-337.85	0.60	-132.12
R50	CMS356	0.03	8.49	-1.38	1.75	32.75	302.16	0.28	144.53
R56	CMS78	1.14	2.86	-0.16	2.44	32.12	174.80	-0.55	65.00
R56	CMS52	1.76	-3.74	0.62	3.21	-105.02	-376.60	-1.16	-203.87
R56	CMS148	-0.36	-0.89	-0.76	0.75	-65.63	-304.10	0.90	-114.49
R56	CMS356	-2.53*	1.74	0.30	-6.37	138.52*	505.91*	0.81	251.98*
SE		1.02	5.02	0.92	2.12	56.35	237.68	1.05	116.12

* and ** significant at 0.05 and 0.01 level of probability respectively

Ordination with PCA was used to determine if there is any structure related to agronomic performance, GCA and SCA regarding multivariate characters. According to combining abilities two principal components accounted for 62% of variability in the GCA and SCA of entries. Ordination in biplot was based on discrimination of entries by multivariate GCA and SCA for measured characters. Traits with higher weight in principal components could discriminate entries effectively to exert multivariate selection. Combining ability of data, seed number (SN), oil yield (OY) and seed yield (SY) had the highest weight in principal component 1, so this component could discriminate entries according to the traits. It can be seen that R23 x CMS78 with highest SCA for SY located further along in the positive direction of its vector (Fig. 1). Two other hybrids with high SCA located near the vector but with a different distance from the vector. The closer one (R21 x CMS52) has a higher SCA. On the other hand,

Table 6. Mean values of agronomic traits in the crosses

Restorer	CMS	Growth Period	Plant Height	Head Diameter	1000 Seed weight	Seed Number /head	Seed yield	Oil Content	Oil Yield
R19	CMS78	106.00	179.63	19.10	70.38	547.26	2,578.35	43.84	1,136.02
R19	CMS52	104.50	192.48	18.05	70.13	710.49	3,241.68	45.37	1,459.63
R19	CMS148	110.00	172.80	18.48	70.50	733.06	3,480.02	43.48	1,503.25
R19	CMS356	106.75	170.88	17.48	75.63	430.36	2,150.01	42.97	915.35
R21	CMS78	103.50	191.25	17.80	59.25	726.07	2,851.68	47.62	1,372.05
R21	CMS52	105.75	194.03	21.78	65.25	965.61	4,200.02	44.33	1,862.48
R21	CMS148	106.25	187.65	17.30	68.75	827.29	3,700.02	44.74	1,663.71
R21	CMS356	109.00	199.13	19.80	82.38	396.79	2,175.01	42.70	928.24
R23	CMS78	106.50	181.48	18.45	77.63	794.72	4,105.02	47.80	1,961.08
R23	CMS52	106.75	200.63	17.15	74.75	594.69	2,920.02	47.68	1,397.32
R23	CMS148	106.75	179.58	18.05	74.38	640.10	3,188.35	46.80	1,502.43
R23	CMS356	117.00	186.40	18.98	84.00	482.57	2,585.01	43.07	1,116.19
R26	CMS78	105.00	181.98	19.05	70.25	579.93	2,678.35	47.59	1,276.24
R26	CMS52	103.75	183.13	19.20	70.63	756.54	3,558.35	47.15	1,674.47
R26	CMS148	105.00	167.33	18.30	67.75	761.51	3,416.68	45.48	1,539.42
R26	CMS356	107.00	176.90	19.38	84.25	401.20	2,365.01	41.69	991.57
R50	CMS78	107.00	196.65	17.30	72.75	699.02	3,365.02	44.21	1,486.29
R50	CMS52	103.50	173.70	19.95	70.50	787.11	3,683.35	43.57	1,608.07
R50	CMS148	102.75	179.13	20.30	67.13	717.11	3,208.35	44.32	1,414.19
R50	CMS356	107.75	196.90	17.33	82.50	543.13	3,083.35	41.39	1,287.25
R56	CMS78	108.25	192.48	17.73	69.00	716.80	3,268.35	47.96	1,571.39
R56	CMS52	108.25	187.15	19.55	70.13	645.72	3,011.68	46.64	1,402.79
R56	CMS148	107.00	179.08	17.15	66.63	665.91	2,976.68	48.44	1,434.26
R56	CMS356	107.75	190.98	18.53	70.13	625.89	3,021.68	45.74	1,397.14
LSD5%		3.53	17.81	3.23	7.72	195.7	819.5	3.74	414.4

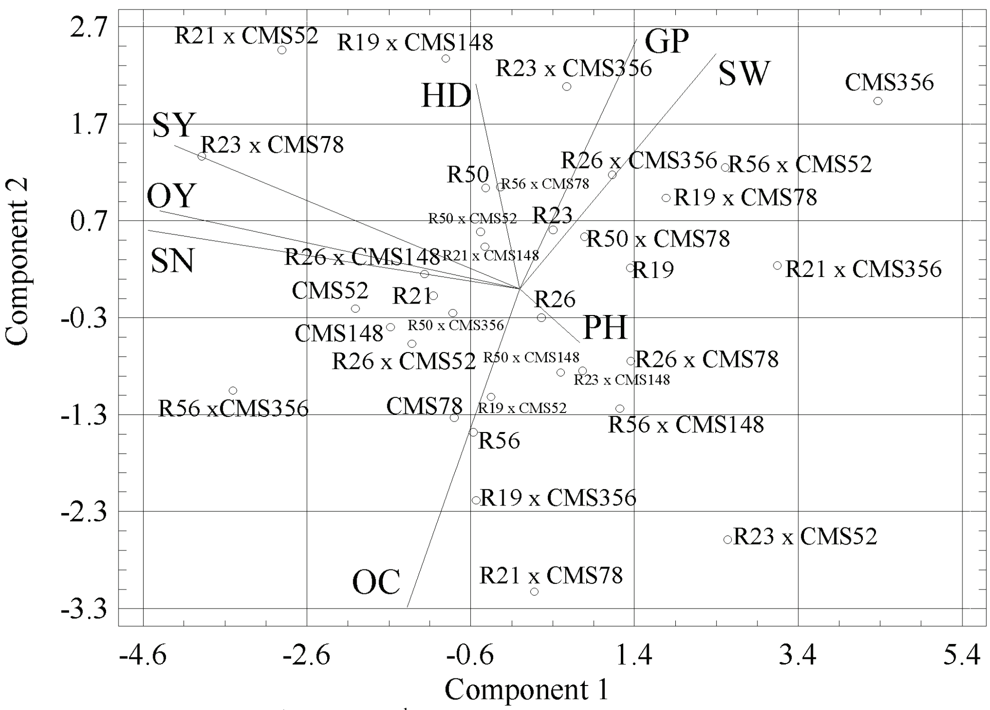

Fig. 1. Biplot of the 1st and the 2nd principal component for general and specific combining abilities.

the lowest SCA belongs to R23 x CMS52, and, logically, it is located further along in the inverse direction of its vector followed by the next high SCA hybrids R21 x CMS356 and R21 x CMS78. This statement is in accordance with de la Vega et al. (1997) who reported the discrimination of genotypes and environment based on their interactions. Acute angles for SY, OY and SN indicated positive associations between them. 1000 seed weight (SW), growth period (GP) and head diameter (HD) is closely associated in this respect. So selection should be made according to one of these traits, accompanied by selection according to the associated traits too, and this would allow making multivariate selection on breeding materials. We found that oil content (OC) has not been associated with SY and OY and that there is a strong negative association for SW with GP and HD because of the obtuse angle for their vectors. Among parental lines, CMS356 had the highest GCA for SW and was located in the same direction of its vector with a distance further away from the origin. R56 and CMS78 had higher SCA for OC and were located in the same direction of their vector. Genotypes with values close to the mean of entries were located near the origin. This was the situation for most parental lines.

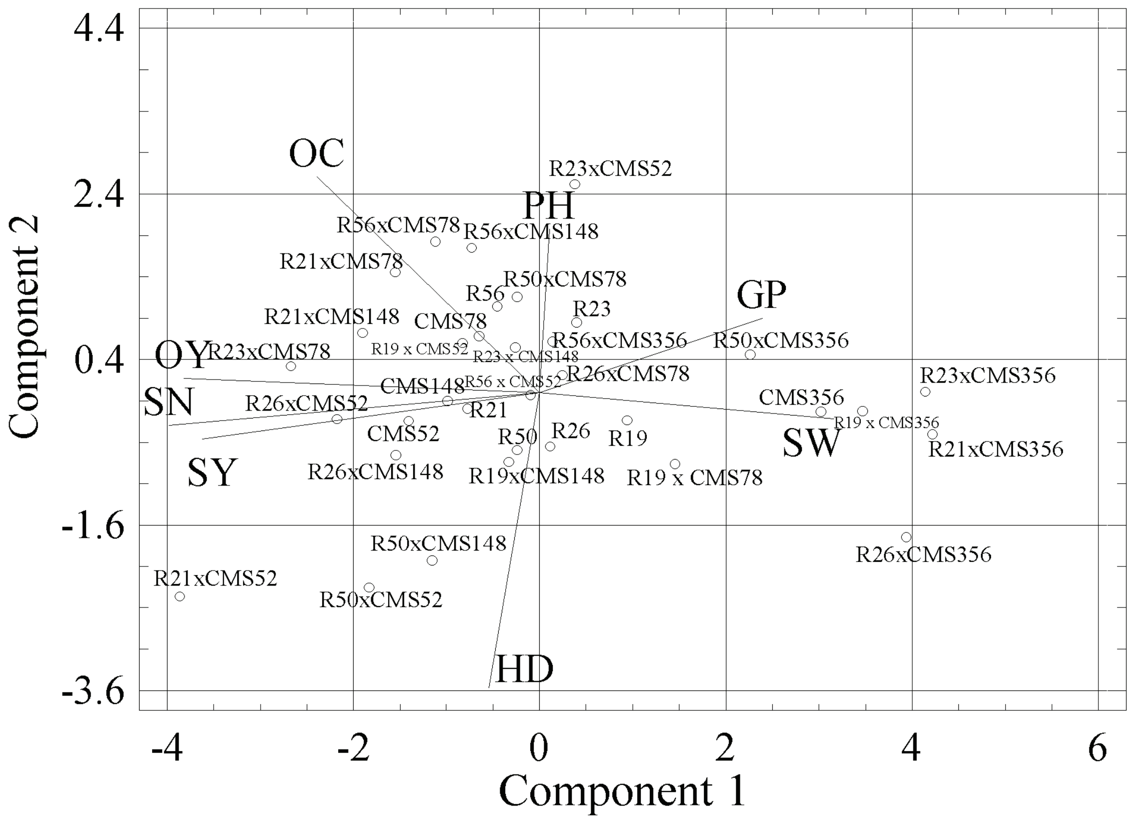

Fig. 2. Biplot of the 1st and the 2nd principal component for agronomic means

Traits with low weight in principal components could not discriminate entries efficiently .This is true for R56 x CMS148 due to its SCA for plant height (PH). Considering agronomic means of SN, OY and SY, these had the highest negative weight in principal component 1, whereas in principal component 2 positive weights of OC and PH and negative weight of HD were higher. A negative association for SN and SW is understood from biplot in Fig. 2. R21 x CMS52 and R23 x CMS78 were located further along in the positive direction of SY vector because of their high seed yields. Hybrids with a low seed yield located on the inverse side of biplot ordination of entries might be influenced by the presence of multivariate effects. For example, ordination of R26 x CMS52 with a lower seed yield than R21 x CMS 148 is not in agreement with the statements, and, in fact, it resulted from the effect of the GP. So, multivariate reactions could cause problems in the ordination of entries, which are felt by breeders considering multivariate selection, but PCA biplots generate equilibrium ordination due to different traits which could be used for precise selections. In this experiment, if it is desired to select an early mature hybrid with a higher oil yield and oil content using Fig. 2, R23 x CMS78 would be a good choice. Association and discrimination behavior of biplot in Fig. 2 is the same as that mentioned for Fig. 1 but the ordination of entries is slightly different. Except R21 x CMS356, all other combinations of R21 are on the left of the biplot in the same direction as that of their parents, except CMS356. It can be seen that R21 has a stronger positive effect on related hybrids than CMS356, and that agronomic means could discriminate genotypes more effectively than SCA data. Also, CMS 356 has a strong increasing effect on its crosses with all 6 restorer lines considering their SW and a decreasing effect on their SY, OY, SN and OC. R56 x CMS356 with higher SCA for SY has a SY close to the mean of entries and SCA was not able to show its real performance. So these biplots provide more useful information to the breeder, PCA based on agronomic data alone could be used for determining the real performance of entries considering multivariate traits.

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New sunflower hybrids tolerant of Tribenuron-Methyl

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ABSTRACT
Discovery of a tribenuron-methyl resistant wild *Helianthus annuus* L. population (ANN-KAN) created an opportunity for expansion of a sunflower herbicide resistance breeding program. The aim of this work was the creation of sunflower hybrids resistant to tribenuron-methyl. Creation of tribenuron-methyl resistant hybrids would enable the use of a wider palette of herbicides for sunflower, more efficient chemical control of *Cirsium arvense* and more economically profitable post-emergence control of some annual broad-lea weeds in sunflower. Original populations SURES-1 and SURES-2 are homozygous for resistance to tribenuron-methyl. F_1 generations produced from the crossings are completely resistant to tribenuron-methyl, pointing to the dominant way of inheritance of this trait. Studies on the exact number of genes controlling the resistance are in progress. Tribenuron-methyl resistance was transferred from original populations into a number of mother and restorer inbred lines of cultivated sunflower. These inbred lines could enable creation of a number of hybrids resistant to tribenuron-methyl. Hybrids NS-H-2017-SU, NS-H-2018-SU and NS-H-2019-SU are resistant to doubled application dose of tribenuron-methyl. Agronomical characteristics of these hybrids are on the level with the leading conventional sunflower hybrids.

Key words: hybrid – sunflower – tolerance – tribenuron-methyl.

INTRODUCTION
The main aim of plant breeding is to develop new varieties and hybrids to meet the needs of people and domestic animals. Due to the rapid growth of the human population, loss of arable land, global climate change, and water supply problems, the production of sufficient amounts of food will be a challenge in the future. The increase of yields of cultivated plants requires not only the development of new, more productive genotypes but the advancement of growing technology as well. Plant breeding for tolerance to herbicides covers both of these aspects.

The development of plants with herbicide tolerance has been made possible by the latest insights into the mechanism and target place of herbicide action at the molecular level and by the development of new biotechnology methods. In the 1990s, a number of crop genotypes resistant to herbicides have been developed as a result (Table 1). Although it is theoretically possible to develop a plant tolerant of any kind of herbicide, only combinations of major economically important crops and herbicides possessing favorable characteristics (glyphopsate, glufosinate ammonium, sulfonylurea, imidazolinones, etc.) have found an actual commercial application (Malidza et al., 1999).

Table 1. Year of first registration of herbicide tolerant crops (Malidza et al., 1999).

Year	Company	Crop
1992	Cyanamid	IMI, IR, IT Maize
1992	Du Pont	STS Soybeans
1995	Calgene	BXN Cotton
1995	AgrEvo	Liberty Link Canola
1996	Monsanto	Roundup Ready Soybeans
1996	Monsanto	Roundup Ready Canola
1997	Monsanto	Roundup Ready Cotton
1997	AgrEvo	Liberty Link Maize
1997	AgrEvo	Liberty Link Soybeans
1999	Monsanto	Roundup Ready Maize

The initial stages of plant breeding for herbicide resistance did not include any work on sunflower. Crop species for which herbicide-tolerant genotypes had been developed began to be grown more widely thanks primarily to the improved economy of their production. A result of this was a decrease of area in sunflower in South and North America, where the new technologies had been accepted without any legal limitations. Additionally, weed killing herbicides are developed less rapidly in sunflower than in the rest of field crops. Weeds cause significant yield losses in sunflower due to a lack of effective herbicides for the suppression of broadleaf weeds and use after crop emergence. The currently existing chemical measures are ineffective against large-seeded broadleaf weeds, while the present soil herbicides are often not effective enough in the suppression of small-seeds weed species, especially in years with rainfall deficits occurring after herbicide application (Malidza et al., 2004). All this prompted sunflower researchers to begin working on the crop's tolerance to herbicides. The first major breakthrough came when Al-Khatib et al. (1998) found a population of wild *Helianthus annuus* L. (ANN-PUR) originating from Rossville, Kansas (USA) that was resistant to imidazolinone-based herbicides. Once the genetics of the resistance were studied and understood (Miller and Al-Khatib, 2000; Jocić et al., 2001), this population was used to develop the first sunflower hybrids tolerant of imidazolinone herbicides. These were developed in the USA in 2003 and Serbia and Turkey in 2004 (Jocić et al., 2004).

The discovery in Kansas, USA of a wild *Helianthus annuus* L. (ANN-KAN) population (Al-Khatib et al., 1999) resistant to a sulfonylurea herbicide (tribenuron-methyl) opened up the possibility of expanding the scope of sunflower breeding for tolerance to herbicides. The present study was aimed at the development of sunflower hybrids possessing tolerance of tribenuron-methyl. The introduction of such hybrids provides multiple benefits, including a broadened range of available herbicides in sunflower, more effective control of Canada thistle (*Cirsium arvense*), and greater cost-efficiency in the suppression of some annual broadleaf weeds after sunflower emergence (Zollinger, 2003; Malidza et al., 2006).

MATERIALS AND METHODS

The herbicide Granstar 75 WG was used in the study in two doses, the normal, recommended one (30 g/ha) and twice that (60 g/ha). In the latter years of the program, another herbicide was also used in the study to test the tolerance of the newly developed hybrids. This was Express 50-SX (500 g/kg tribenuron-methyl), a new and improved tribenuron-methyl-based herbicide manufactured by Du Pont. Express 50-SX was applied at 45 g/ha (standard dose) and 90 g/ha (double dose).

The sources of genes for tolerance to tribenuron-methyl were the populations SURES-1 and SURES-2. SURES-1 is a population of B lines obtained from the cross HA 424/3HA 406 // HA 89/ ANN-KAN, while SURES-2 is a population of restorer lines originating from the cross RHA377/3 RHA 392 // RHA 376/ ANN-KAN (Miller and Al-Khatib, 2004). Of cultivated sunflower genotypes, we used the self-pollinated B lines HA-26, VL-A-8 and HA-48 for crosses with SURES-1 and the restorer lines RHA-583, RHA-SES and RHA-N-49 for crossing with SURES-2.

The tolerance of SURES-1 and SURES-2 towards tribenuron-methyl was tested in the greenhouse during September through December 2000. In parallel with this, initial crosses were made between the two populations and the self-pollinated lines chosen for the study. During the 2001 growing season, the tolerance of the resultant F_1 generations was tested under field conditions using the double dose of tribenuron-methyl. After determining the mode of inheritance, pedigree selection was employed, with each inbred generation being treated with the double dose of Granstar 75-WG (60 g/ha). The most tolerant plants from the most tolerant progenies were selected for further breeding work. Treatment with herbicides was performed at the stage of 2-6 leaves using the knapsack sprayer Solo, 350 l/ha of water and a pressure of 2 bars. Twenty days after the treatment, phytotoxicity was assessed visually on a scale of 0 to 100% (0% - no symptoms, 100% - complete plant necrosis). Thanks to the use of a greenhouse, three inbred generations were obtained per year, which enabled us to develop the first experimental hybrids as early as 2004 and to test the general (GCA) and specific (SCA) combining abilities of the newly developed restorer lines. The testing was done using line x tester method (Singh and Choudhary, 1976). The comparative trial was carried out on a well-prepared chernozem soil at the Rimski Sancevi Experiment Field of the Institute of Field and Vegetable Crops using a randomized block design with three replications. The planting dates were optimal, intensive cultural practice was implemented during the growing season, and harvesting was done manually. The best hybrid combinations were selected and tested for tolerance to tribenuron-methyl and performance characteristics in a network of small-plot trials in 2005.

RESULTS AND DISCUSSION

Tribenuron-methyl is a herbicide that inhibits the acetolactate synthase enzyme (ALS), which is responsible for the synthesis of the amino acids valin, leucine and isoleucine. It is also one of the oldest sulfonylurea herbicides in existence (Ferguson et al., 1985) and has been among the most important herbicides in small grains for the past two decades. In Serbia, it is used in wheat crops and is the active ingredient of the Granstar 75-WG formulation (75% tribenuron-methyl) (Mitic, 2004). According to

Kolkman et al. (2004), the SURES-1 and SURES-2 populations have been found to contain the Pro197 mutation. This mutation is one of the most common mutations found in crop species tolerant of herbicides inhibiting ALS. It provides several-fold tolerance towards such herbicides compared with the susceptible genotypes. During the 2001 growing season, progenies of the source populations were found to possess full tolerance to tribenuron-methyl, meaning these populations are fully homozygous for this trait. Full susceptibility of the conventional inbred lines was confirmed as well. The F_1 generations exhibited full tolerance along with slight chlorosis, but there was absolutely no lagging behind in growth of any sort relative to the control treatment, which indicates the dominant mode of inheritance of tolerance to Granstar 75-WG. Determining the genetic basis of herbicide tolerance is a very sensitive kind of research. The first requirement is to use the double dose of the active ingredient. Environmental factors have a great influence on the expression of herbicide tolerance, as does the genetic basis of the lines receiving the tolerance genes. Because the donor populations possess many traits characteristic of the source population of wild *Helianthus annuus*, the determination of the genetics of the tolerance requires prior development of inbred lines tolerant of tribenuron-methyl. Pedigree selection was used to develop 52 inbred lines from crosses between SURES-2 and the restorer lines RHA-583, RHA-SES and RHA-N-49 as well as 46 female inbreds obtained by crossing SURES-1 and the lines Ha-26, VL-A-8 and Ha-48. All these self-pollinated lines are tolerant of the double dose of tribenuron-methyl, since the herbicide was applied at the 2-6-leaves stage in each generation during their development. Besides the herbicide tolerance, the newly developed selfed lines also have other favorable agronomic characteristics (most importantly tolerance to *Phomopsis helianthi*), as these were selected for these characteristics as well during the selection process.

The development of these lines also enabled the development of the first hybrids tolerant of tribenuron-methyl. The GCA and SCA of the new lines were tested and then the experimental hybrids were developed in 2004. All the hybrids were tested for performance characteristics and resistance to the common diseases and treated each year with the double dose of tribenuron-methyl. Based on the results, three of the hybrids were chosen for commercial production.

Due to the large volume of this research program, the present paper shows only the results for the newly developed SU hybrids NS-H-2017-SU, NS-H-2018-SU and NS-H-2019-SU. Table 2 shows the results produced by the three hybrids in two years of testing. The main requirement these hybrids must meet is to have a sufficient level of tolerance to tribenuron-methyl. What this means in concrete terms is that they have to be able to withstand the double dose of the standard, recommended dose of the active ingredient per unit area without showing any signs of phytotoxicity or any significant losses of yield or yield components. The results achieved by our hybrids have shown that they have a sufficient level of tolerance, as there were no statistically significant yield losses or reductions in the other studied traits in the treatment with the double dose of tribenuron-methyl relative to the treatment in which no herbicide was used (Table 2). Additionally, there were no visible signs of phytoxicity either. The only thing observed was that there was some slight chlorosis seven days after the treatment, but these symptoms disappeared completely after two weeks. The second important condition the new hybrids have to fulfil is to have good performance characteristics in addition to tolerance to tribenuron-methyl. Thus, they have to have a high yield potential, a high oil content, and resistance to the common diseases so as to be able to compete with the standard sunflower hybrids used in commercial production. The check hybrids in our trials were NS-H-111, the leading sunflower hybrid in Serbia, and NS-H-43, which is a hybrid that domestic sunflower growers are well familiar with, as it has been present in Serbian sunflower production for a considerable number of years already. The results of the trials have shown that the new SU hybrids are completely on a par with the standard ones in terms of performance. The performance of NS-H-2017-SU and NS-H-2019-SU completely matched that of the class-leading NS-H-111 in terms of seed yield, oil content and oil yield, while NS-H-2018-SU performed as well as NS-H-43 despite being two weeks earlier in terms of maturation (Table 2).

Our results indicate that the new SU hybrids NS-H-2017-SU, NS-H-2018-SU and NS-H-2019-SU will find their niche in the domestic sunflower market very soon. This has been confirmed by their results in the official variety trials of the Serbian Variety Commission and their subsequent registration in the Serbian Variety List.

The source populations SURES-1 and SURES-2 are homozygously tolerant of tribenuron-methyl. The F_1 generations produced in the program are completely tolerant of tribenuron-methyl, indicating the presence of the dominant mode of inheritance. Studies to determine the exact number of genes controlling this resistance are in progress. Resistance to tribenuron-methyl has been transferred from the source populations to a number of female and self-pollinated sunflower lines. This makes it possible to develop a larger number of hybrids tolerant of tribenuron-methyl. The hybrids NS-H-2017-SU, NS-H-2018-SU and

NS-H-2019-SU are tolerant of twice the recommended dose of tribenuron-methyl per hectare and are also as good as the leading sunflower hybrids in the domestic market in terms of agronomic performance.

Table 2. Mean values of several traits in tribenuron-tolerant sunflower hybrids

Hybrid	Treatment	Plant height (cm)	Maturity (days)	Seed yield (kg/ha)	Oil content (%)	Oil yield (kg/ha)
NS-H-2017	Untreated	176.43	122.5	4 265.33	46.36	1 976.43
	Tribenuron-methyl (45 g/ha)	178.29	123	4 230.57	46.25	1 956.20
NS-H-2018	Untreated	161.40	110.4	3 702.26	47.18	1 747.45
	Tribenuron-methyl (45 g/ha)	162.30	109.9	3 806.34	48.53	1 847.28
NS-H-2019	Untreated	189.55	127.8	3 926.57	48.30	1 896.53
	Tribenuron-methyl (45 g/ha)	188.75	127.5	4 157.52	49.25	2 046.11
NS-H-43	Untreated		129	3 938.04	46.49	1 830.35
NS-H-111	Untreated		123	4 258.12	48.53	2 066.36
			LSD	476.93	4.27	260.65

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Genetic improvement of oil quality in sunflower mutants under water stressed conditions

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ABSTRACT

The objective of the present research was to study 30 mutants, induced by gamma irradiation among a population of M6 sunflower mutant lines and to identify molecular markers associated with different seed quality traits. The experiments were performed in two environments (greenhouse and field) under well-watered and water-stressed conditions. Experiments consisted of three blocks, and each block was split into two main plots (well-watered and water-stressed). The seed quality traits studied were: protein content (PC), oil content (OC), palmitic acid (PA), stearic acid (SA), oleic acid (OA) and linoleic acid (LA). In both environments and both conditions, a large genetic variation was observed between mutant lines and some mutants presented higher values for seed quality traits in comparison to original line. Two mutants M6-862-1NI and M6-826-2 showed the most important values for OC and OA in comparison with the original line AS613 under all conditions. The results revealed the efficiency of gamma-irradiation for inducing genetic variation in sunflower for seed quality traits. Multiple regression analyses showed that some AFLP markers are associated with several traits. The most important were E40M59-5 and E33M49-5 markers, with more than 30% of phenotypic variance for OC and PA under water stressed condition. There were several markers associated with quality traits in both well-watered and water-stressed conditions. Some other markers were specific for only one trait or a given water treatment. The markers which were associated with different traits could be used for marker-assisted selection.

Key words: AFLP – gamma irradiation – genetic variation sulfonylurea – oil quality sulfonylurea – water stress – sunflower.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the four major annual world oilseed crops which is cultivated for edible oil. In commonly grown sunflower cultivars, the oil contains a high proportion (about 90%) of unsaturated fatty acids; oleic (C18:1) and linoleic (C18:2) acids. The remaining 10% corresponds to the saturated fatty acids palmitic (C16:0) and stearic (C18:0) acids (Garcés et al. 1989).

Mutagenesis has been successfully used for developing variation in the fatty acid profile of sunflower and mutants with an altered fatty acid content have been developed, such as high oleic acid mutant Pervenets (Soldatov, 1976), mutants 275HP and CAS-5 with high palmitic acid content (Ivanov, 1988; Osorio et al., 1995), CAS-12 with high palmitic and oleic acids (Fernandez-Martinez et al., 1997), mutants CAS-8, CAS-4 and CAS-3 with medium to high stearic acid (Osorio et al., 1995) and CAS-14 with very high stearic acid (Fernández-Moya et al., 2002). Using molecular markers in selecting genotypes with desirable traits through marker-assisted selection has been proved to be effective in plants. Identification of markers associated with important traits in a group of genotypes through multiple regression analysis offers an alternative means and has been adopted in several plant species. As an example, Vijayan et al. (2006) identified several ISSR markers associated with yield traits in mulberry. The aim of this study was to identify the interesting mutants for oil quality and to detect the molecular markers associated with oil quality traits.

MATERIALS AND METHODS

A population of gamma rays mutant lines (M6) coming from AS613 genotype was developed in our laboratory (Sarrafi et al. 2000). Among this population, 30 mutants showed morphological differences in comparison with 'AS613', which were used in our experiments.

The quantitative traits were evaluated in two environments: greenhouse under controlled conditions and field. Each experiment was conducted with two conditions: well watered and water stressed. Both experiments consisted of three blocks, and each block was split into two main plots (well- watered and water stressed). Protein content (PC), oil content (OC), palmitic acids (PA), stearic acid (SA), oleic acid (OA) and linoleic acid (LA) were measured by FOSS NIR System 6500 (Foss Analytical, Denmark).

Genomic DNA of AS613 and mutant lines were isolated from two-week old seedlings according to the method of extraction and purification presented by Fulton et al. (1995). Different *MseI* / *EcoRI* primer combinations were used for AFLP genotyping. The AFLP procedure was conducted as described by Al-Chaarani et al. (2004). AFLP bands were scored from the gel as presence (1) or absence (0).

The variability among the mutants for the studied traits was tested through ANOVA. A simple correlation between the quantitative traits was calculated. The association between AFLP markers and quantitative traits was obtained through stepwise multiple regression analysis, where each quantitative trait was considered as a dependent variable while the AFLP marker was treated as an independent variable (Vijayan et al. 2006).

RESULTS

Analysis of variance showed significant variability among mutant lines for all seed quality traits in two environments under well-watered and water-stressed conditions. The effect of water-stress was significant for PC, OC and SA in the greenhouse as well as for SA in the field (data are not presented).

Table 1. Seed quality traits and genetic gain (GG)[1] of sunflower mutants in two experiments: greenhouse (1) and field (2) under well-watered (WW) and water-stressed (WS) conditions.

Mutants	Exp.	PC[2]		OC		PA		SA		OA		LA	
		ww	ws	ww	ws	ww	ws	ww	ws	ww	ws	ww	ws
AS613	1	19.2	18.6	45.7	45.3	5.7	5.3	3.3	3.3	33.3	38.8	59.6	53.9
	2	22.4	21.4	37.6	38.9	5.5	6.0	5.8	4.2	34.2	29.4	53.2	60.3
M6-826-2	1	24.6	24.1	43.6	47.0	5.0	4.7	4.5	4.6	61.0	72.2	29.9	20.5
	2	23.4	19.7	34.1	41.8	5.0	4.8	8.2	9.4	43.2	48.0	42.4	37.7
M6-133-2	1	24.3	23.1	39.9	43.3	5.4	5.1	3.7	4.2	42.1	46.1	49.6	46.0
	2	27.3	22.1	33.7	33.8	7.2	7.2	5.4	5.5	22.7	21.9	64.8	68.1
M6-375-1	1	21.7	20.0	39.0	39.1	5.1	4.9	3.9	3.8	41.5	41.4	50.1	50.1
	2	24.8	23.7	30.3	35.0	5.3	5.1	5.7	5.4	33.2	37.9	55.0	51.0
M6-862-1NI	1	16.4	16.5	54.8	54.3	6.3	5.9	1.8	1.8	36.5	35.8	61.3	53.4
	2	20.8	18.5	43.3	50.1	5.7	5.2	4.0	3.8	19.9	23.1	55.6	52.6
M6-186-1	1	20.8	18.6	44.2	47.8	6.2	6.8	2.7	2.8	28.5	18.5	62.9	72.4
	2	23.4	16.9	37.3	37.9	7.1	7.5	5.0	5.0	23.4	19.9	64.8	67.5
M6-653	1	21.8	21.8	45.2	45.3	5.1	4.9	3.8	4.3	43.4	44.5	48.5	46.8
	2	24.9	20.4	33.8	36.2	9.5	6.3	3.9	5.1	26.7	30.3	58.3	58.2
M6-641-2	1	20.2	21.8	40.8	43.4	5.3	5.6	3.7	4.0	41.9	36.9	49.6	54.6
	2	20.7	16.4	35.2	38.2	5.8	7.4	3.8	3.2	26.7	17.4	63.6	71.9
GG	1	5.1*	5.5^{ns}	9.1*	9.0*	0.6*	1.5*	1.2*	1.3*	27.7*	33.4*	3.3^{ns}	18.5*
	2	4.9*	2.3^{ns}	5.7*	11.2*	4.0*	1.5*	2.4*	5.2*	9.0*	18.6*	11.6*	11.6*

[1]GG: Genetic gain calculated as the differences between the best mutant and original line (*AS613*). Values are presented for the original line AS613 and for 7 selected mutants which present the highest values.

[2]PC, Protein Content; OC, Oil Content; PA, Palmitic Acid content; SA, Stearic Acid content; OA, Oleic Acid content; LA, Linoleic Acid content.

*: significant at 0.05 level, ns: non significant.

Negative significant correlation was observed between OC and PC, OA and LA, PA and SA and SA with LA, whereas correlation between SA and OA was positive (data are not presented). Some mutants presented high values for more than one trait (M6-186-1, M6-862-1NI, and M6-826-2) and some others just for one trait (Table 1). Some mutants presented significant differences with the original line AS613 for several traits. Genetic gain (GG) calculated as the difference between the best mutant and original line was significant for all the studied traits except for PC under water-stressed condition in all environments and for LA under well-watered condition in greenhouse (Table 1).

Table 2. Main markers associated with the seed quality traits in sunflower mutants.

			Well-watered						Water-stressed		
Trait	Exp.	Marker	P	R^2 (%)	M1- M2	Trait	Exp.	Marker	p	R^2 (%)	M1- M2
OC	1	***E40M59-5***	*	15.2	-5.51	OC	1	***E40M59-5***	***	35.3	-8
OC	2	***E40M59-5***	**	24.4	-7.1	OC	2	***E40M59-5***	*	18.7	-5.74
PC	1	*E33M50-17*	**	29.8	-2.91	PC	1	*E33M50-17*	**	21.7	-2.97
PC	1	*E37M50-14*	*	17.1	-2.2	PC	1	*E31M48-4*	*	16.7	2.11
PC	2	***E40M59-5***	*	14.8	3.38	PC	1	***E40M59-5***	*	13.2	3.72
PA	1	*E37M50-14*	*	19.41	0.42	PC	1	*E37M50-6*	**	19.8	-2.57
PA	1	*E31M50-1*	**	22.2	-0.38	PC	2	***E40M59-5***	*	17.3	2.96
SA	1	***E40M59-5***	**	22.8	1.02	PC	2	*E31M50-1*	*	16.4	1.58
SA	1	*E31M50-1*	**	23.7	0.54	PA	1	*E37M50-14*	*	16.2	0.34
SA	2	*E37M50-14*	**	22.8	-1.41	PA	1	***E40M59-5***	*	13.7	-0.50
SA	2	*E33M50-17*	**	23.2	-1.42	PA	2	*E37M50-6*	*	20.7	-0.27
SA	2	*E37M48-8*	*	18	-1.54	PA	2	*E33M49-5*	**	33.2	1
SA	2	*E31M50-1*	**	23.4	1.16	SA	1	***E40M59-5***	**	22.5	1.2
OA	1	*E37M50-14*	**	21.9	-9.52	SA	2	*E37M50-14*	**	23	-1.47
OA	1	*E37M48-8*	**	23	-11.64	SA	2	*E37M50-6*	**	21.3	-1.26
OA	1	*E33M50-17*	**	22	-9.55	SA	2	*E37M48-8*	*	18.3	-1.54
LA	1	*E37M50-14*	**	23	9.75	OA	1	*E37M50-6*	**	18	-6.37
LA	1	*E37M48-8*	**	23.6	11.46	OA	1	*E37M50-14*	*	16.6	-6.75
LA	1	*E33M50-17*	**	22.9	9.71	OA	2	*E37M50-6*	**	29.4	-8.43
LA	2	*E37M48-8*	*	14.2	6.88	OA	1	*E33M59-12*	**	19.7	6.66
LA	2	*E40M59-1*	**	24.8	-6.29	OA	2	*E37M48-8*	**	21.1	-9.9
						LA	1	*E37M50-6*	**	22.2	7.27
						LA	1	*E33M59-12*	**	19.9	-6.95
						LA	2	*E37M48-8*	*	19.7	9.87
						LA	2	*E37M50-6*	**	30.4	8.84

PC, Protein Content; OC, Oil Content; PA, Palmitic Acid content; SA, Stearic Acid content; OA, Oleic Acid content; LA, Linoleic Acid content . 1; greenhouse, 2; field. M1-M2: difference between two marker classes as revealed by analysis of variance of trait by marker genotype

In total 34 and 31 AFLP markers associated with the quantitative traits were identified in greenhouse and field, respectively (Table 2). More than 61% of the detected markers were identical in two experimental environments. Some markers were associated with different traits (for example, E33M50-17, associated with PC, SA, OA and LA) and some others were common across water treatments such as E40 M59-5 associated with OC under well-watered and water-stressed conditions (Table 2).

The results showed that E40M59-5 and E33M49-5 markers are the most important ones in water–stress conditions with more than 30% of phenotypic variance for OC and PA.

DISCUSSION

Significant genetic variation observed among mutant lines for the studied traits revealed the efficiency of gamma-irradiation for inducing genetic variation in sunflower for seed quality traits. Some mutant lines presented advantages over AS613 for different traits. Two mutant lines M6-862-1NI and M6-826-2 showed important values of OC and OA, respectively, in comparison with the original line AS613 in two environments under both conditions (well-watered and water-stressed). These mutant lines could be used in breeding programmes to improve seed oil content under water stress growth conditions. On the other hand, the mutant line M-862-1NI showed the maximum value for PA only under well-watered condition and a low value under water-stressed condition. This mutant is a sensitive genotype under water stress. The significant negative correlation between PC and OC in our mutant lines was observed also by Mahmood et al. (2006) in *Brassica juncea*.

Our results show that some AFLP markers are associated with several traits and some others are specific for only one trait or a given water treatment (Table 2). The phenotypic variance explained by each marker (R^2) was important, ranging from 13.7 to 35.3%. E40M59-5 marker was associated with OC in two environments under both conditions (well-watered and water-stressed). The latter marker is the most important marker in this study as it is associated with some other traits (PC, PA and SA) in well-watered and water-stressed conditions. Thus E40M59-5 could be used in marker assisted selection programmes in sunflower. Also E31M50-1 marker was common between PA and SA under well-watered condition in greenhouse. Overlapping QTLs for PA and SA were reported by Burke et al. (2005). Several common markers for OA and LA were found in each environment. This can be explained by correlation between OA and LA as well as by a specific gene of Δ12-desaturase which converts oleic acid into linoleic acid in grains and modifies fatty acids composition, as reported in sunflower and soybean (Garcés et al., 1989; Heppard et al., 1996). E37M48-8 and E37M50-6 were also common in two environments for OA and LA. E37M50-6 was a specific marker for water-stress while E37M48-8 was non-specific for well-watered and water-stressed conditions. Markers associated with different traits in both water treatments could be used for marker-assisted selection in both environments. Other markers, which are specific for one water treatment but associated with different traits or specific for a trait, could be useful for a given water treatment.

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Estimation of genetic diversity of sunflower single cross hybrids using principal component analysis

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ABSTRACT
In order to study genetic diversity of sunflower single cross hybrids through using principal component analysis, a North Carolina Design I experiment was conducted in a randomized complete block design in three replications at Agricultural and Natural Research Station of Khoy. In 2005, six fertility restorer male lines were crossed with 18 cytoplasmic male sterile (CMS) female lines. Each male line was crossed with three different female lines to make two sets with nine hybrids in each set. In 2006, Single cross F_1 hybrids were planted for studying genetic diversity. Data were collected from agronomic traits including: flowering initiation, seed filling period, maturity, plant height, head diameter, 1000 seed weight, seed numbers per head, harvest index, oil content, seed yield and oil yield. Survey results showed that 3 first components explained variability of all the data (77.20 %). Traits including seed numbers per head, harvest index, oil content, seed yield and oil yield were the main parts of first component while second component was affected mainly by flowering initiation, maturity and plant height. Seed filling period, head diameter, seed yield and oil yield were the important parts of third component. In all, the first component with a large amount of total variance and high correlation with traits including seed yield, oil yield, seed numbers per head, oil content and harvest index could be appropriate and useful for grouping and selecting superior single cross hybrids.

Key words: genetic diversity sulfonylurea – hybrid sulfonylurea – principal components analysis – sunflower.

INTRODUCTION
In recent years, many sunflower single cross and three-way-cross hybrids have been produced in Iran, and a selection of the best of them in regional preliminary experiments is very important task. Multivariate statistical methods that can create relationships between cultivar traits, can help to group cultivars and make it easy to select them on the basis of biplot and triplot diagrams. The principal components analysis method, through the summarizing of preliminary correlated varieties in the form of independent and limited components, provides the possibility of genotype grouping in the 2D or 3D space (Moghaddam et al., 1994). Some researchers, in order to accelerate the genotype selection for aspects of traits like seed yield have used principal components analysis, a method which is also useful for the reduction of the selection cost and in the preliminary stage of cultivar selection (Spranaaij and Bos, 1993). Kroonenberg et al. (1995) also used this method, with three-way-cluster analysis, for the separation of genotypes in limited bunches for their manipulation and Cheres and Knapp (1998) have used the efficient grouping of this method for the evaluation of genetic diversity in sunflower germplasm and determination of ancestral relationship. De La Vega et al. (2001) have used principal components analysis for the determination of interaction among different sunflower cultivars with cultural media and for the consideration of indirect selection possibility of yield in these media and Ghaffari (2003) used this methodologies for rapid screening of 121 sunflower cultivars and hybrids, so that logical cultivar grouping through the influence from agronomic traits could be used as an efficient factor in superior and early mature hybrid selection. Finally, Zeinalzadeh-Tabrizi and Ghaffari (2005) have used this method for the survey of genetic diversity of sunflower genotypes These authors employed in their experiment, commercial cultivars such as Azargol, Record, Armavirski and Hysun33 well-distinguished from three-way-cross hybrids through the high seed and oil yield by first component. In biplot and triplot diagrams, designed on the basis of facts obtained from principal component analysis, trait influence on the genotype grouping in different vector forms and the position of every genotype on the basis of selected component type are shown.

Length of any vector shows the weight of that vector in the creation of distinguished groups and is correlated with the amount of component for the related traits.

Through the designing of a vertical line from a genotype location to trait vector, genotypes can be compared. In effect, the further the distance of conjunction of the line with the origin, the more the diversion of genotype yield from others (Chapman et al., 1997). Any angle among the vectors in these diagrams shows their correlation (Kroonenberg, 1997). With this kind of relationship among the agronomic traits and related vectors in formed diagrams, is possible to group experimental genotypes logically. We can use this method as a way for surveying genetic diversity of evaluated materials and also for the selection of superior genotypes in preliminary experiments.

The aim of this study was the use of principal component analysis for the obtention of biplot and triplot diagrams, that can be used for surveying genetic diversity and selection of superior sunflower single cross hybrids under study, , and for the determination of relationships between traits.

MATERIALS AND METHODS

This experiment was conducted at Agricultural and Natural Resources Research Station of Khoy, Iran (44° 58´ N, 38° 33´ E) during 2004 and 2005. The minimum, average and maximum annual temperature of this station are, respectively, -30, 12.5 and 42°C and the average annual rainfall is 292.6 mm. Plant material used in this experiment were sunflower single cross hybrids obtained from crosses of 6 male restorer lines (R line) with 18 female cytoplasmic male sterile (CMS or A) lines which have been produced at Agricultural and Natural Resources Research Station of Khoy in 2003. In each set, were included 3 R lines crossed with different 3 CMS lines and their developed 9 hybrids. Hybrids containing common male parent were counted as half-sib family. Mating had been done as a nested design in North Carolina Design I plan, as such, CMS lines have been nested inside restorer lines. F_1 hybrids obtained from crosses were planted for the principal components analysis and to survey genetic diversity in 2005.

Surveyed traits were: flowering initiation, seed filling period, maturity, plant height, head diameter, 1000 seed weight, seed number per head, harvest index, oil content, seed yield and oil yield. Correlation among the varieties was done through the use of SPSS software and the principal components analysis employing Statgraphics, biplot and triplot diagrams being designed. On the basis of the characteristics of every hybrid, and the direction and angle of the related vectors, the position of 18 hybrids in biplot and triplot diagrams have been clarified, and, on that basis, the range of the existing diversity and methods of grouping obtained have been considered.

Hybrids characteristics are shown in charts below.

First Set		
Hybrid	**R line**	**A line**
A		CMS_{28}
B	R_{43}	CMS_{128}
C		CMS_{346}
D		CMS_{330}
E	R_{27}	CMS_{78}
F		CMS_{328}
G		CMS_{336}
H	R_{34}	CMS_{52}
I		CMS_{148}

Second Set		
Hybrid	**R line**	**A line**
J		CMS_{344}
K	R_{56}	CMS_{260}
L		CMS_{32}
M		CMS_{222}
N	R_{25}	CMS_{96}
O		CMS_{356}
P		CMS_{356}
Q	R_{32}	CMS_{196}
R		CMS_{376}

RESULTS AND DISCUSSION

Equation of every 3 first components has been shown in Table 2. For example, equation of first component is:

Z1= -0.05 FI-0.32 SFP-0.23 MA+0.06 PH+0.16 HD-0.35 SW+0.41 SN+0.32 HI+0.35 OC+0.36 SY+0.40 OY

Table 1. Variance of components in principal component analysis method

Component number	Eigen value	Variance percentage	Cumulative percentage
1	5.10	46.40	46.40
2	2.01	18.32	64.73
3	1.37	12.47	77.20
4	0.99	9.03	86.24
5	0.74	6.72	92.96
6	0.38	3.49	96.46
7	0.20	1.88	98.35
8	0.13	1.19	99.54
9	0.03	0.32	99.86
10	0.01	0.12	99.99
11	0.00	0.00	100

Table 2. Structure of first 3 components for agronomic traits

Traits	Component 1	Component 2	Component 3
Flowering Initiation (FI)	-0.05	0.57	0.09
Seed Filling Period (SFP)	-0.32	-0.01	0.32
Maturity (MA)	-0.23	0.50	0.29
Plant Height (PH)	0.06	0.36	-0.06
Head Diameter (HD)	0.16	-0.44	0.49
1000 Seed Weight (SW)	-0.35	0.03	0.23
Seed Number per Head (SN)	0.41	0.06	0.17
Harvest Index (HI)	0.32	0.00	-0.37
Oil Content (OC)	0.35	0.28	-0.16
Seed Yield (SY)	0.37	0.07	0.46
Oil Yield (OY)	0.40	0.14	0.31

Through carrying out the principal components analysis and grouping the genotypes on the basis of the quantity of two first components, it was clarified that genotypes, on the basis of trait weight in every component, obtain a special position in correlation with the agronomic trait vector and are scattered according to the correlation of considered traits with components and according to the quantity of trait under study (Figs. 2 and 3).

This kind of genotype scattering in provided vectors can afford at least the possibility of fast rejection or selection of main parts of genotypes and this could be useful in preliminary evaluations. Because genetic materials used each experiment are different, genotype orientation around the related vectors of agronomic traits will depend on the correlations obtained in every experiment and trait weight in the formation of every component. Because of that, the method of selection in every experiment will be different from the other. The use of this method is not limited to sunflower crop, and like other multivariate methods, it can be used in other products.

In this study, on the basis of general waypoint we have reached the conclusion that the first component obtained from principal components analysis with 46.40 percent of the total variance and high correlations with traits like seed numbers per head, harvest index, oil content, seed yield and oil yield, can be used in an efficient way in the fast selection and screening of genetic materials in the initial stages. Genotypes which are in the right half of the biplot diagram and around the vector related to the seed yield, besides having a high seed yield, have a high oil yield, oil content and number of seed per head. Selection on the basis of these genotypes with a short maturity period is beneficial to the effort to achieve the most important aims of production of sunflower hybrids seed programs.

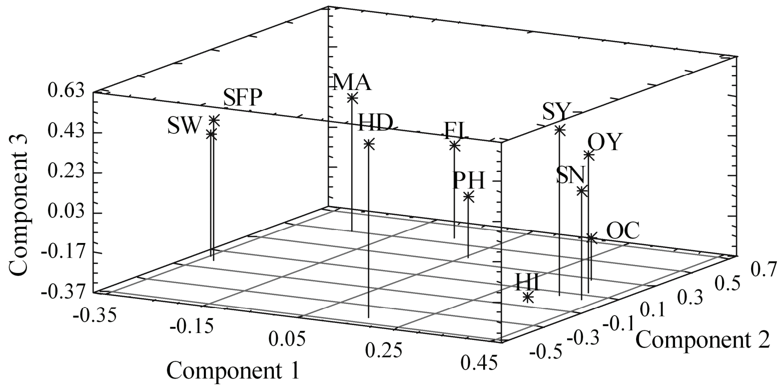

Fig. 1. Diagram of first three component weights for agronomic traits on sunflower single cross hybrids. Abbreviations are given in table 2.

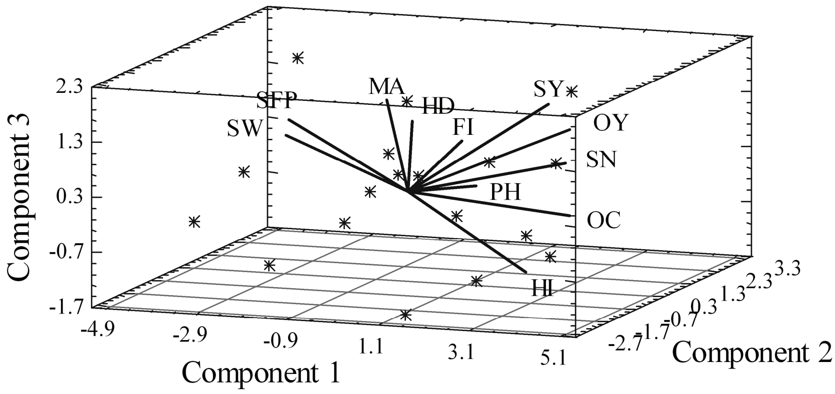

Fig. 2. 3D diagram for position of sunflower genotypes and trait vectors in principalcomponent analysis method. Abbreviations are given in table 2.

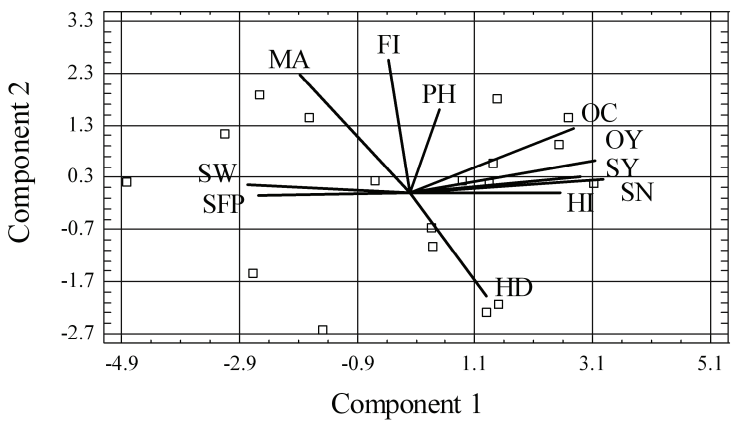

Fig. 3. Biplot for position of sunflower genotypes and trait vectors in principal components analysis method. Abbreviations are given in Table 2.

Table 3. Correlation among agronomic traits of sunflower single cross hybrids and components of principal component analysis

Traits	Flowering Initiation	Seed Filling Period	Days to Maturity	Plant Height	Head Diameter	1000 Seed Weight	No. of seeds per Head	Harvest Index	Oil Content	Seed Yield	Oil Yield
FI	1										
SFP	-0.115^{ns}	1									
MA	0.648**	0.600**	1								
PH	0.133^{ns}	-0.146^{ns}	0.113^{ns}	1							
HD	-0.379^{ns}	-0.142^{ns}	-0.479*	-0.200^{ns}	1						
SW	0.142^{ns}	0.579*	0.439^{ns}	0.143^{ns}	-0.064^{ns}	1					
SN	-0.043^{ns}	-0.548*	-0.314^{ns}	0.079^{ns}	0.346^{ns}	-0.811**	1				
HI	-0.073^{ns}	-0.641**	-0.490*	0.121^{ns}	0.083^{ns}	-0.572*	0.583*	1			
OC	0.073^{ns}	-0.523*	-0.187^{ns}	0.339^{ns}	-0.002^{ns}	-0.651**	0.717**	0.614**	1		
SY	0.034^{ns}	-0.417^{ns}	-0.200^{ns}	0.142^{ns}	0.529*	-0.449^{ns}	0.863**	0.388^{ns}	0.558*	1	
OY	0.066^{ns}	-0.504*	-0.221^{ns}	0.205^{ns}	0.388^{ns}	-0.561*	0.903**	0.493*	0.750**	0.965**	1
Component 1	-0.107^{ns}	-0.735**	-0.530**	0.140^{ns}	0.376^{ns}	-0.788**	0.932**	0.727**	0.790**	0.820**	0.896**
Component 2	0.804**	-0.016^{ns}	0.713**	0.509**	-0.623**	0.050^{ns}	0.082^{ns}	0.005^{ns}	0.395^{ns}	0.095^{ns}	0.199^{ns}
Component 3	0.106^{ns}	0.381^{ns}	0.344^{ns}	-0.077^{ns}	0.580*	0.275^{ns}	0.198^{ns}	-0.431^{ns}	-0.189^{ns}	0.535*	0.364^{ns}

ns, * and **: Non significant, significant at 5 % and 1 %, respectively.

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Relationship between genetic distance and heterosis based on quantitative traits and SSR markers in sunflower

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ABSTRACT

The objective of this study was to determine the relationship between SSR based genetic distance (GD) of new NS sunflower inbred lines for most important agronomic traits and heterosis. Twenty three sunflower inbred lines (twenty restorer lines and three female lines used as testers) were selected based on their diverse genetic background for plant height, head diameter, thousand seed mass, oil content, seed yield per plant and oil yield per plant. Significant heterosis was observed in hybrid combinations for all examined traits except oil content. Genetic distance between pairs of tested sunflower inbred lines ranged from 0.13 to 0.8. There was no significant positive correlation between genetic distance and mid- and better-parent heterosis, specific combining ability and mean value in any of the examined traits for all 60 hybrids. A highly significant negative correlation was found between GD and mean oil percentage ($r=-0.33$ $p<0.01$). However significant correlations between GD and better-parent heterosis for thousand seed weight were found for hybrids of the tester line HA-19 ($r=0.43$ $p<0.05$) and between GD and mid-parent heterosis for plant height for hybrids of the tester line HA-26 ($r=0.47$ $p<0.05$). Although GD was generally a poor predictor of heterosis, better results are obtained if hybrid combinations for each tester and each trait are analyzed separately.

Key words: correlations sulfonylurea – genetic distance sulfonylurea – heterosis sulfonylurea – hybrid performance – sunflower.

INTRODUCTION

Identification of parental combinations that produce hybrids of superior yield is the most important step in the breeding program of sunflower (*Helianthus annuus* L.). However, developing hybrids is a costly and long term process, as it is necessary to cross a lot of inbred lines and evaluate hybrids in field trials. Therefore, only a limited number of hybrids among all possible crosses can be tested. Utilisation of genetic distance for predicting hybrid heterosis has been of great interest to breeders. The efficiency of hybrid breeding programs could be increased if the inbred lines *per se* could be screened and the superior crosses predicted before field evaluation (Melchinger et al., 1990).

Studies of genetic diversity in relation to hybrid performance have been undertaken in several crops. Investigations in corn, *Zea mays* L. have shown that the genetic diversity of parents was significantly correlated with hybrid performance and that yield heterosis could be predicted using molecular markers (Smith et al., 1990; Betran et al., 2003; Rief et al.; 2003; Schrag et al., 2006). Conversely, weak correlations have been reported between genetic distance and hybrid performance and heterosis in oilseed rape, *Brassica napus* L. (Diers et al., 1996), pepper, *Capsicum annuum* L. (Geleta et al., 2004), faba bean, *Vicia faba* L. (Zeid et al., 2004), and alfalfa, *Medicago sativa* L. (Riday et al., 2003).

Different sunflower gene pools have been studied for their genetic diversity with different marker systems (Tersac et al., 1993; Gentzbittel et al., 1994; Berry et al., 1994; Zhang, 1995; Hongtrakul, 1997, Cheres and Knapp, 1998; Yu et al., 2002; Tang and Knapp, 2003; Pankovic et al., 2004; Solodenko et al., 2005). However, the literature data on the predication of sunflower heterosis and hybrid performance by marker based genetic distance of the parental lines is scarce (Tersac et al., 1994; Cheres et al., 2000). Cheres et al. (2000) used AFLP markers and found a significant correlation between GD and seed yield, but genetic distance was generally a poor predictor of hybrid performance. The objective of this study was to determine the association between SSR based genetic distance of new NS sunflower inbred lines for most important agronomic traits and heterosis.

MATERIALS AND METHODS

Twenty three sunflower inbred lines (20 restorer lines and three female lines used as testers) were selected based on their diverse genetic background for examined agronomic traits. The selected restorer lines (labeled R-1 through R-20) are new inbred lines developed in the breeding program of the Oil Crops Department, of the Institute of Field and Vegetable Crops, in Novi Sad, Serbia. Female lines used as testers (HA-48, HA-26 and HA-19) are commercial lines with good combining abilities.

Female lines were crossed with restorer lines to produce all possible combinations of F_1 hybrids using the line x tester method (Singh and Choudhary, 1976). Seeds of the 60 F_1 hybrids produced and their parents were sown in a breeding nursery of the Oil Crops Department, of the Institute of Field and Vegetable Crops. The experimental design was a randomized block system with four replications.

Plant height (PH), head diameter (HD), thousand seed weight (TSW), oil content (OC), seed yield per plant (SY) and oil yield per plant (OY) were used for quantitative characterization of 23 parental lines and their 60 F_1 hybrids. Plant height and head diameter were measured at the end of flowering. Seed yield was measured by harvesting the middle row of each plot by hand. Seed samples from each plot were analyzed for oil content by nuclear magnetic resonance.

Analysis of variance and specific combining abilities (SCA) for quantitative traits were performed using the line x tester method (Singh and Choudhary, 1976). Heterosis was determined as follows:

Mid-parent heterosis (MPH) (%) = $((F_1\text{-MP})/\text{MP})*100$

Better-parent heterosis (BPH) (%) = $((F_1\text{-BP})/\text{BP})*100$

where, F_1 is the F_1 performance, MP = $(P_1+P_2)/2$ in which P_1 and P_2 are the performances of inbred parents and BP is the betterparent value (Geleta et al., 2004). Significance of heterosis was determined by the t-test (Kraljevic-Balalic et al., 1991).

Genomic DNA of 23 parental lines was extracted following the modified method of Dellaporta et al. (1983). The 15 SSR sunflower primers used in the study were: ORS 1, ORS 5, ORS 7, ORS 8, ORS 10, ORS 12, ORS 14, ORS 16, ORS 31, ORS 37, ORS 47, ORS 66, ORS 78, ORS 509 and ORS 595 (Tang et al., 2002). The selected primers have previously revealed DNA polymorphism of sunflower NS breeding material (Pankovic et al., 2004; Terzic et al., 2006). Fragments were separated using 2% agarose and 6% denaturing polyacrylamide gels. DNA polymorphism between two inbred lines was estimated by comparison of amplified fragments. Jaccard coefficient (J) of similarity was calculated according to Staub et al. (2000). Genetic distances (GD) among the 23 parental lines were estimated according to Spooner et al. (1996) as GD = 1-J.

Values of genetic distance as measured by SSR markers were correlated with MPH and BPH to estimate their relationship. Correlations were done for F_1 combination from each tester line separately and all tester lines.

RESULTS AND DISCUSSION

Parental lines and 60 F_1 hybrids were evaluated in field trials for plant height, head diameter, thousand seed weight, oil content, seed yield per plant and oil yield per plant. There was a great variation among inbred lines and hybrids, respectively (Table 1). The mean values of the hybrids were significantly higher than the parental lines for plant height, head diameter, thousand seed mass, seed and oil yield per plant.

Table 1. Mean values, standard error of the means and coeficient of variation (V) for the sunflower parental lines and their F_1 hybrids

Trait	**Female line**		**F_1 hybrid**		**Restorer**	
	Mean	V	Mean	V	Mean	V
Plant height (cm)	157.77±0.87	20.10	201.88±0.45	45.19	141.48±0.36	51.43
Head diameter (cm)	18.69±0.01	19.47	22.48±0.02	36.98	14.21±0.01	66.04
Tousand seed weight (g)	50.66±0.21	9.49	54.33±0.07	8.62	34.49±0.25	22.07
Oil content (%)	46.77±0.10	6.11	47.36±0.09	5.42	47.90±0.13	6.12
Seed yield (g per plant)	35.38±0.65	10.15	57.05±0.58	14.60	12.24±0.20	38.69
Oil yield (g per plant)	16.46±0.33	4.66	26.99±0.26	14.83	5.91±0.05	42.46

The heterotic effect was observed in all examined traits, except oil content (Table 2). The mean values of hybrids were between parental means for oil content and both parental lines were selected for high oil quantity. The highest effect of heterosis (MPH) was observed for oil yield per plant (143.77%) followed by seed yield per plant (142.04%).

Table 2. Mean values and range of heterosis (%) for six quantitative traits of the 60 F_1 sunflower hybrids (PH=plant height, HD=head diameter, TSW=thousand seed weight, OC=oil content, SY=seed yield per plant and OY=oil yield per plant)

Heterosis		PH	HD	TSW	OC	SY	OY
MPH							
	Mean	35.36**	37.17**	21.37**	0.06	142.04**	143.77**
	Range	15.32-66.86	17.24-66.56	0.20-65.22	-7.12-9.72	60.17-249.44	55.77-247-24
BPH							
	Mean	21.28**	19.00**	3.45*	-0.46	62.04**	64.10**
	Range	-4.01-42.70	0.74-47.81	-18.27-34.85	-9.98-6.98	29.14-130.92	34.72-125.32

**significant at P=0.05 , *significant at P=0.01

Analysis of fifteen SSR markers detected 44 alleles, with an average polymorphism PIC= 45.3%. The number of alleles per locus ranged between 2 and 5, with a mean of 2.93. Genetic distance between pairs of tested sunflower inbred lines ranged from 0.13 (HA-19 vs. HA-48 and R-12 vs. R-18) to 0.8 (HA-19 vs. R-18) (data not presented).

The relationship between genetic diversity based on SSR markers of all inbred lines and their hybrid performance depended on the trait examined. Correlation coefficients between GD and parental means, SCA and heterosis were not significant for the most examined traits (Table 3). The only significant correlation was a negative one, between GD and mean oil content ($r=-0.33$ $p<0.01$). For plant height, correlation between GD and heterosis was positive but not significant ($r=0.232$ and 0.172). Similar results were obtained for thousand seed weight (0.226 and 0.245).

Table 3. Correlation between genetic distance (GD) and mid- (MPH) and better-parent heterosis (BPH), specific combining ability (SCA) and mean values (MV) for each trait in sunflower hybrids (PH=plant height, HD=head diameter, TSW=thousand seed weight, OC=oil content, SY=seed yield per plant and OY=oil yield per plant).

	PH	HD	TSW	SY	OC	OY
GD vs. MPH	0.232	0.096	0.226	-0.213	-	-0.202
GD vs. BPH	0.172	0.101	0.245	-0.067	-	-0.071
GD vs. SCA	0.020	0.099	0.090	-0.159	-0.154	-0.178
GD vs. MV	-0.115	-0.102	0.071	0.021	-0.330**	-0.103

$r_{(0,05)}$=0,25, $r_{(0,01)}$=0,325

Correlation between genetic distance and heterosis was not significant for most of the examined traits. The poor correlation might be due to several causes. SSR markers used in this study were chosen solely for their high PIC values. Charcosset et al. (1991) and Bernardo et al. (1992) suggested that genetic distance cannot accurately predict hybrid performance unless the DNA markers used in the analysis were linked to the genes affecting the trait. Therefore, the 60 F_1 hybrids were divided into three groups according to the parental tester line and correlation of the GD with hybrid performance, and heterosis within the groups was examined for all six traits. Only significant correlations were found between GD and better-parent heterosis for thousand seed mass for hybrids with the tester line HA-19 ($r=0.43$ $p<0.05$) and between GD and mid-parent heterosis for plant height for hybrids with the tester line HA-26 ($r=0.47$ $p<0.05$) (Fig. 1). In these two cases hybrid heterosis increased linearly with increased GD between parental lines. However, the correlations obtained were too low to be of any predictive value.

Tersac et al. (1994) described relationships between heterosis and enzymatic polymorphism of 39 sunflower populations. The correlation coefficients for all enzyme systems were too low to be used as predictors of the general combining ability, but when enzyme systems were analyzed separately, four of them turned out to be useful markers for breeding purposes. Cheres et al. (2000) have used 360 AFLP markers and found that although genetic distances were significantly correlated with hybrid seed yield and percent of heterosis for seed yield ($r=0.79$ and 0.76), hybrid performance varied greatly among hybrids of inbreds with similar genetic distance (GD). Zeid et al. (2004) pointed out that the lack of association between heterosis and genetic dissimilarities for inter group hybrids might be explained by absence of crosses between related parents i.e. by the absence of variation for parental relatedness: all crosses have unrelated parents.

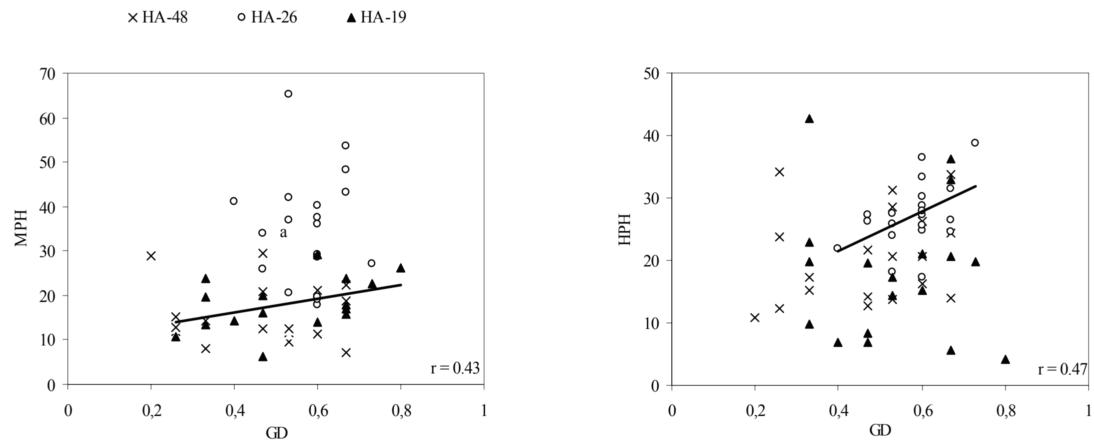

Fig. 1. Plots of genetic distance vs. mid-(MPH) and better-parent heterosis (BPH) for plant thousand seed weight (left) and plant height (right) of sunflower hybrid combinations ($r_{(0,05)}$=0.42, $r_{(0,01)}$=0.54).

The results of this study confirm that GD generally correlates poorly with heterosis and specific combining abilities. Previous studies in various crop species such as corn, pepper, alfalfa, wheat, and rapeseed also showed low correlations of GD with heterosis (Melchinger et al., 1990; Diers et al., 1996; Geleta et al., 2004; Zeid et al., 2004; Riday et al., 2003). Although genetic distance is a poor predictor of hybrid performance, our results indicate that better results are obtained if hybrid combinations for each tester and each trait are analyzed separately. Our further field trials for identification of sunflower heterotic performance will be planned on prior information on genetic distance of inbreds, obtained by more molecular markers, involving the ones associated with QTLs for examined traits.

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