PHYSIOLOGY

DO CELL WALL PROTEINS AFFECT THE SETTING OF GRAINS AND THEIR POTENTIAL WEIGHT IN SUNFLOWER?

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ABSTRACT

Physiological bases of grain setting and potential grain weight are still partially understood in sunflower. There are evidences that grain number (GN) and weight (GW) are sensitive to environmental conditions immediately before flowering (R5) and during grain filling. Additionally, it has been pointed out that a better knowledge about the growth of maternal tissues of grains (ovary/pericarp) will improve the understanding of GN and potential GW settings, highlighting the key role of expansins (proteins controlling plant cell wall loosening). This study aimed to evaluate the impact of ectopic applications of cell wall proteins, including expansins, on GN and GW in sunflower. Two contrasting grain weight genotypes were sown in a split plot design with three replicates at the Agricultural Research Station (UACh), Chile. Cell wall proteins were extracted from sunflower seedlings and they were applied on the capitula at R4 or after 10 days of flowering (R5). Two control treatments (without proteins and only buffer applications) were also assessed. Extracts of proteins were assessed by SDS-PAGE and by mapping and database searches. Fresh and dry weight of ovaries and grains (dissecting pericarp and embryo) were recorded from R3 to physiological maturity. At harvest, GN, GW and oil concentration were measured. Proteins applied at R4 increased ($P<0.05$) GN (20%) and GW (30%) in both genotypes. Lower impact was found under applications at 10 days after flowering. Remarkably, oil concentration of grains was not affected ($P>0.05$). These results support that the growth of maternal tissues before R5 affects GN and potential GW in sunflower highlighting the likely key role of expansins.

Key Words : expansins, ovary, kernel, grain yield

THE GENETICS AND EVOLUTION OF SOLAR TRACKING

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ABSTRACT

The heliotropic movement of sunflower shoots, also known as solar tracking, is a dramatic example of a diurnal rhythm in plant growth. The shoot apex continuously tracks the sun's position in the sky as it changes from east at dawn to west at dusk over the course of the day. At night, the apex reorients back to an eastward orientation. As a sunflower reaches reproductive maturity, these cycles dampen, and disks predominantly maintain an eastward orientation at anthesis. Though these phenomena have long been observed, the developmental and molecular mechanisms by which external cues and internal rhythms are integrated to produce these diurnal patterns of growth are largely unknown. We have taken developmental and natural variation approaches at multiple evolutionary scales to understand the physiology, genetics, and diversity of these traits. Manipulative studies implicate the circadian clock as a driver of nocturnal reorientation and as a regulator of mature head orientation. Through phenotyping an association mapping panel of 280 cultivated sunflower lines with time-lapse imaging in the field, we have described ample diversity in the mean and variance of the diurnal phase of solar tracking movements and the orientation of mature disks, and we have identified several SNPs significantly associated with multiple solar tracking parameters. Finally, a survey of other diploid *Helianthus* species reveals that solar tracking is common among annuals and perennials with broad distributions but not found in basal rosette perennials of the southeastern US, suggesting this trait likely evolves as a component of a resource-acquisitive ecophysiological syndrome.

Key words: heliotropism, movement, circadian clock, phototropism, natural variation, association mapping

INTRODUCTION

Plants experience daily predictable cycles in the availability of resources and in the occurrence of environmental stresses. To cope with these oscillating environmental conditions, many aspects of plant growth, development, and physiology are adapted occur with diurnal rhythms such that peak activity coincides with the most favorable portion of a 24-h period. Although fluctuations of external cues like light or temperature may be the sole drivers of these diurnal plant traits, more often internal rhythms driven by the endogenous circadian clock also play an essential role in jointly coordinating these biological cycles (Alabadi and Blazquez, 2009; Harmer, 2009). Clock regulation is especially important for activities that must anticipate the availability of resources or the onset of environmental pressures, as waiting to directly experience these factors as cues may leave plants with insufficient time to mount fully effective responses, (e.g., activating metabolic or physiological defenses against diurnally active herbivores and pathogens; Wang et al., 2011).

Solar tracking, or heliotropism, of the growing stems of the common sunflower, *Helianthus annuus*, is perhaps the most conspicuous example of a diurnal growth trait in the plant kingdom (Vandenbrink et al., 2014; Kutschera and Briggs, 2016). During the day, the stem grows such that the shoot apex continuously reorients to remain normal to incident sunlight throughout the day, thus tracing a path from facing east at dawn to facing west at dusk (Fig. 1). The stem also reorients at night such that the shoot apex once again faces east in anticipation of dawn (Fig 1). Both movements appears to be largely driven by growth through irreversible cell expansion, as sunflower lacks specialized motor organs known as pulvini that promote reversible, turgor-driven heliotropism of leaves in other systems (Koller, 2001).

Heliotropic movement begins soon after sunflower seedlings begin expanding their true leaves but then slows as plants approach anthesis, at which point the plants stop tracking and maintain an easterly orientation until senescence (Shibaoka and Yamaki, 1959; Lang and Begg, 1979). This final point has been subject to a long-running misconception. For centuries, many authors have erroneously stated that mature heads do track the sun (e.g., Gerarde, 1597; Kircher, 1667; Koller, 2011), leading those who have then failed to observe floral heliotropism to dismiss the phenomenon entirely (Gerarde, 1597; Meehan, 1884; Kellerman, 1889). However, seminal studies corrected the literature by publishing photographic evidence of the daily movements of young plants (Schaffner, 1898, 1900).

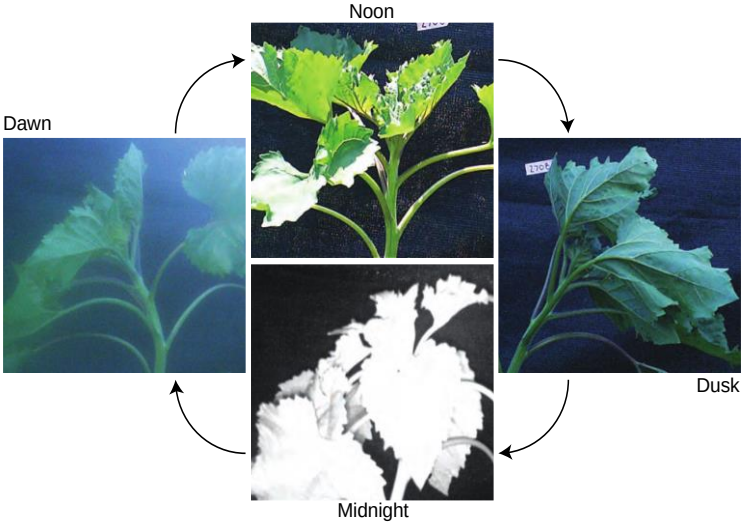

Fig. 4: Solar tracking and noctural reorientation of the sunflower stem. East was to the left and west to the right of the plant filmed in this series. Midnight photo taken with infrared LED flash built into camera.

Although the solar tracking of sunflower stems has been observed for centuries, the biological mechanisms that govern this behavior and the evolutionary history of the trait have received little attention (Shibaoka and Yamaki, 1959; Vandenbrink et al., 2014; Kutschera and Briggs, 2016). For instance, while we know a moving light source is a critical driver of heliotropic bending, how this signal from the changing relative position of the sun is perceived and how it leads to differential growth of lateral stem segments paced to the sun's east-to-west trajectory are largely unknown. Moreover, although an endogenous rhythm has been implicated in the regulation of solar tracking because plants rotated 180º take several days to fully match their growth to the new orientation (Shibaoka and Yamaki, 1959), the nature of this rhythm and its interactions with environmental signals are also not understood. Finally, the evolutionary history of solar tracking, the abundance of natural variation in this trait, as well as the ecological functions of heliotropism and the eastward orientation of mature disks have been little explored.

Here, we discuss what is known with respect to the first two physiological questions and also report several aspects of our work in progress that aims to address the final evolutionary question using a variety of approaches. First, we review previous studies on the regulation of solar tracking. Then, we report an initial assessment of natural variation in the timing of nocturnal reorientation using a recently generated association mapping panel of cultivated

sunflower. Finally, we discuss how our preliminary survey of diversity in solar tracking within the genus indicates how solar tracking may serve as part of a larger ecophysiological syndrome adapted for resource acquisition.

POSSIBLE MECHANISMS REGULATING SOLAR TRACKING

Surprisingly little has been published on the physiological mechanisms underlying solar tracking despite the long period over which this trait has been recognized (Schaffner, 1898, 1900; Vandenbrink et al., 2014; Kutschera and Briggs, 2016). Given that sunflowers do not have pulvini, it is very likely that the movements of solar tracking stems are due to asymmetric growth on the two sides of the stem, as has been reported for the petioles of leaves undergoing rhythmic 'sleep movements' (Pfeffer, 1903). The coincident timing with which solar tracking and leaf cell expansion cease at anthesis has also led several authors to infer that solar tracking is a growth mediated process (Lang and Begg, 1979; Koller, 2001). However, unlike rhythmic leaf movements, the initiation of solar tracking requires cues from the environment. Strongly directional light is clearly required to drive stem movements during the day. Plants grown under stationary overhead light in greenhouses or growth chambers do not track (Shell and Lang, 1976; B. Blackman, S. Harmer, personal observation), and several investigators have reported instances in which young plants have failed to track on cloudy or rainy days (Schaffner, 1898; Shibaoka and Yamaki, 1959). It is very likely that the daily east-to-west movements of sunflower plants is auxin-mediated and is initiated by the well-studied phototropin signaling pathway (Fankhauser and Christie, 2015).

However, no strong directional light source exists in nature that can explain the stereotyped west-to-east nocturnal reorientation of sunflower stems. We suggest that this directional movement at night in anticipation of dawn may be generated by circadian regulation of growth pathways. Several lines of evidence support this possibility. For instance, resetting of solar tracking movements takes several days when plants are experimentally rotated 180º during the night (Shibaoka and Yamaki, 1959). In addition, under long day photoperiods, the speed of stem movement must be and is substantially more rapid at night than during the day for the shoot apex to face east by dawn (Schaffner, 1898, 1900; Vandenbrink et al., 2014; Kutschera and Briggs, 2016). Finally, in some instances developing buds have been observed to achieve their eastward orientation well ahead of dawn (Shell and Lang, 1975; B. Blackman, personal observation). These observations all suggest involvement of an endogenous mechanism in solar tracking.

We therefore predict that the circadian clock provides the mechanistic basis for the endogenous rhythms that interact with directional light signaling and other environmental cues to drive solar tracking and nocturnal reorientation. In particular, we expect that the circadian clock drives diurnal rhythms in the abundance or activity of light signaling components and hormones that drive differential stem growth (Foster and Morgan, 1995; Millar and Kay, 1996; Jouve et al., 1999; Covington et al., 2008). The circadian clock may also gate how responsive plants are to these stimuli at particular times of day, following a paradigm that has been developed through the study of plant growth and organ expansion in controlled environmental conditions (Covington and Harmer, 2007; Nozue et al., 2007; Arana et al., 2011). We are currently conducting organismal and molecular experiments that will allow us to better understand the physiological mechanisms underlying solar tracking under naturally fluctuating field conditions and, in doing so, to determine whether the circadian clock does in fact play an instrumental role in governing one or more aspects of this fascinating plant growth behavior.

ASSOCIATION MAPPING IDENTIFIES NATURAL VARIANTS ASSOCIATED WITH SOLAR TRACKING

Natural variation can also provide a useful entry point to begin connecting genotype to phenotype and thus to understand the molecular basis of particular traits. We have complemented our ongoing developmental studies by taking an association mapping approach to further characterize the molecular mechanisms that regulate solar tracking. Concerted efforts by the Compositae Genome Project and the Sunflower Genome Consortium over the past decade have produced a panel of 288 lines that harbor ~90% of the common alleles segregating in cultivated sunflower (Kane et al., 2011; Mandel et al., 2011, 2013; Bachlava et al., 2012; Bowers et al., 2012). This panel is a tremendous resource. Because the genotypes are known and the lines are largely inbred and homozygous, any phenotype that can be scored on the panel can be quickly associated with single nucleotide polymorphisms (SNPs). Moreover, because this panel has been thoroughly genotyped by a succession of genomic methods over time with release of whole-genome resequencing data for the whole panel imminent, the genotypic resolution for association mapping is becoming comprehensive and high-resolution (Mandel et al., 2013; Nambeesan et al., 2015).

We have phenotyped the sunflower association mapping panel for solar tracking at a field plot at Morven Farm, VA, a property owned by the University of Virginia Foundation. Because filming all lines concurrently was prohibitively costly and difficult, we planted three replicates per line across a series of fifteen staged plants. Replicates were evenly distributed such that each accession had one replicate grown in the first third of the plantings, one in the middle third of the plantings, and one in the final third of the plantings. For a given replicate, three seeds were sown in a five-gallon paint bucket containing local soil mixed with 10% compost and with several holes drilled in the bottom for drainage. Plants were watered once or twice daily dependent on local conditions and plant size, and thinning was performed two weeks after germination.

Plants were filmed ~5 weeks on average after sowing, during the developmental period after budding but well before anthesis for most accessions. For filming, the buckets were placed in front of a matte black backdrop, and we used Bushnell X-8 trail cameras to capture images every 10 min for 48 to 72 h. The resulting time-lapse videos were visually evaluated for several traits, including the timing of nocturnal reorientation (i.e., the time relative to dusk when the stem first appears to move eastward instead of westward). The compass orientation of heads at anthesis was also scored on all plants. Association mapping was conducted for the means and coefficients of variation for each trait using a mixed-linear model that controlled for population structure and kinship in TASSEL v3.0 (Bradbury et al., 2007; Zhang et al., 2010). Genotypic data for the panel consisted of ~5.8K SNPs previously scored using an Illumina Infinium SNP array (Mandel et al., 2013).

We observed abundant variability in the timing of nocturnal reorientation in the association mapping panel. While the majority of lines began nocturnal reorientation within 30 minutes before or after dusk (mean = -6.2 ± 2.5 min), a notable number of lines began nocturnal reorientation over an hour earlier or later than dusk (Fig. 2A). The variability of this trait within lines also varied among lines. That is, for lines where three replicates were scored, we observed that the standard deviation in the timing of nocturnal reorientation ranged from 2 min to 2 h.

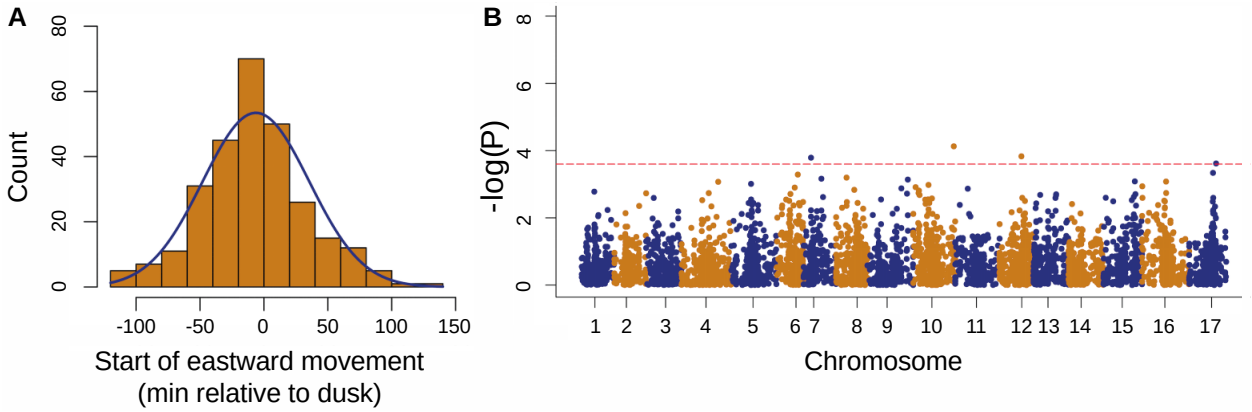

Figure 5: Phentoypic and genetic variation in timing of nocturnal reorientation. (A) Distribution of 288 cultivated sunflower lines scored by time-lapse photography for the time relative to dusk when the stem begins to reorient toward East. (B) Manhattan plot illustrating the significance level of associations tests for ~5.8K SNPs with the timing of nocturnal reorientation. Dashed red line indicates significance threshold after correction for multiple tests.

Association mapping yielded several SNPs significantly associated with variation in the mean timing of nocturnal reorientation (Fig. 2B). The significantly associated SNPs are located in annotated transcripts homologous to a mitochondrial ATP synthase G subunit family protein, NAD(P)H-quinon oxidoreductase subunit L, a DnaJ domain transcription factor, and a DTW domain-containing protein. We also detected several SNPs associated with variability in mature head orientation, including a homolog of the core circadian clock component *LATE ELONGATED HYPOCOTYL* (*LHY*), possibly corroborating a role for the clock in solar tracking traits.

The limited number of significant SNPs observed may reflect the genetic architecture of intraspecific variation in this trait. Traits largely governed by many rare alleles and/or common alleles of moderate effect typically show similar patterns. However, these findings may also reflect the limited sampling of genomic space provided by the current genotypic dataset. We expect the strength of our approach to improve as the full resequencing dataset for the association mapping panel becomes available. That data will be very helpful for determining whether these genes or closely linked genes are best associated with the trait and thus most likely to have a causal influence. Moreover, we expect a sizable portion of the genome is not in strong linkage disequilibrium with any of the SNPs in the current sample, and thus there may be ample potential to detect additional significantly associated polymorphisms.

SOLAR TRACKING: A RESOURCE-ACQUISITIVE ECOPHYSIOLOGICAL SYNDROME TRAIT?

Solar tracking has been most remarked upon and studied in wild and cultivated populations of the common sunflower, *Helianthus annuus*. However, Schaffner also observed

solar tracking of the stems of two other wild *Helianthus* species over 100 years ago (Schaffner, 1898, 1900). These old observations raise several questions. How far back in the sunflower lineage did this behavior evolve? Is solar tracking evolutionarily labile? Does solar tracking demonstrate correlated evolution with other characters as part of a broader ecophysiological syndrome?

To address these questions, we filmed a subset of the diploid *Helianthus* species during the summers of 2014 and 2015 at our field site at Morven Farm outside of Charlottesville, VA, USA. Seeds were scarified and germinated on moist Whatman paper in Petri dishes in the dark for up to 7 days. After one day of light exposure, the seedlings were transplanted into cell packs containing a 1:1 mixture by weight of Fafard 3B soil and calcined clay. Seedlings were raised for up to four weeks in the University of Virginia Greenhouses under 16 h days before transplantation into the ground or into buckets filled with soil at our field site. Stems were filmed for 72 to 96 h during the developmental period after budding but before anthesis. Images captured every 5 or 10 min, and the resulting time-lapse videos were visually evaluated for evidence of tracking.

A revised, generally well resolved phylogeny of diploid *Helianthus* developed through sequencing and analysis of 170 nuclear genes was recently published (Fig. 3; Stephens et al., 2015). When considered on this tree, our preliminary findings show a striking pattern of character evolution for solar tracking. The phylogeny resolves the genus into three majors clades: annuals, erect perennials with widespread distributions in North America, and perennials mostly endemic to the southeastern United States that often grow as basal rosettes. In our diversity survey, we observed solar tracking for all members sampled from both the annual and widespread perennial clades (Fig. 3). We also observed solar tracking for another member of the widespread perennial clade not included in the diploid tree because the species consists of both diploid and polyploid populations, *H. decapetalus*, and Schaffner reported tracking of the polyploid *H. pauciflorus*, which belongs to this clade as well (Schaffner, 1898). In contrast, we did not observe solar tracking for any of the members of the southeastern perennials sampled or for additional closely related but poorly resolved perennial species (Fig. 3). Although some of these taxa do grow as basal rosettes (*H. atrorubens*, *H. radula*, *H. occidentalis*), others do not (*H. floridanus*, *H. mollis*). Thus the pattern we observe cannot be explained solely by constraints on internode elongation during the period of active leaf expansion.

Notably, a recent macroevolutionary analysis reported similar phylogenetic patterns for many leaf economics spectrum and resource use traits (Mason and Donovan, 2015). That is, correlated patterns of evolution were observed such that the annual and widespread perennial clades appear to evolve a correlated syndrome of resource-acquisitive trait values (e.g., deltoid leaves, greater vein length per unit area, higher stomatal conductance). In contrast, the southeastern perennial clade appears to evolve toward a syndrome of resource-conservative trait values (i.e., lanceolate or acuminate leaves, lower vein length per unit area, lower stomatal conductance). If more comprehensive sampling confirms the similar preliminary pattern we observe for solar tracking, then these findings would corroborate the hypothesis that solar tracking serves a critical function in enhancing resource acquisition, a longstanding idea that has been difficult to test empirically. Because we have not been able to grow and film an outgroup to the genus and yet observe tracking of *H. porteri*, the most basally diverging taxon with in the genus, the important question of when and in what lineage solar tracking first evolved remains unresolved. In addition, due to poor resolution of branching events ancestral to the southeastern perennial clade, some uncertainty remains about how

strictly congruent the transition to a resource-conservative ecophysiological syndrome is with the evolutionary loss of solar tracking.

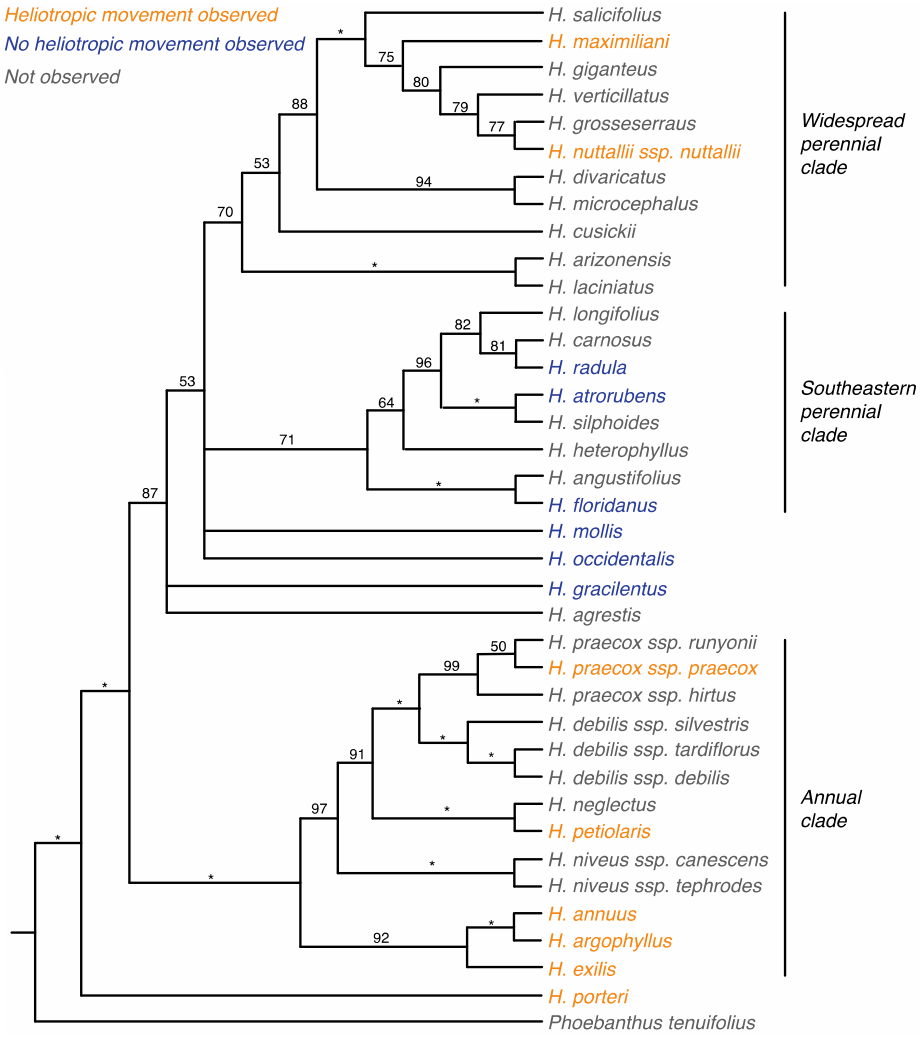

Figure 6: Phylogenetic survey of solar tracking. Species names are colored by trait status (see inset). Figure adapted from Stephens *et al.* 2015. We also observed that a diploid accession of *H. decapetalus*, a member of the widespread perennial clade not included in the species tree does exhibit solar tracking. The species tree was constructed with Maximum Pseudo-likelihood Estimation of the Species Tree v1.4 (MP-EST; Liu *et al.* 2010). Boostrap support provided for nodes, asterisks indicate bootstrap support = 100. Nodes with <50 bootstrap support collapsed.

CONCLUSIONS AND FUTURE DIRECTIONS

It has been extensively shown in several systems under controlled conditions that the interaction of the circadian clock with external signals drives diurnal cycles of light signaling components and hormones that play essential roles in directional plant growth. By focusing on solar tracking as a model system, we are working to determine whether this paradigm also holds true for a growth trait that impacts plant fitness in changing natural environments. Natural variation shows great promise as an experimental means of learning about these underlying mechanisms, and we expect the release of whole genome resequencing data for the cultivated sunflower association mapping panel to enhance these efforts dramatically. In

addition, the diversity in solar tracking that we have observed among *Helianthus* species appears to provide insight into the function of solar tracking as part of an ecophysiological syndrome of evolutionary correlated traits that enhance resource acquisition. Comparative developmental and transcriptomic studies across species that do and do not track may also prove a fruitful means of gaining understanding into the mechanisms that regulate this fascinating plant growth trait.

ACKNOWLEDGEMENTS

Many aspects of this work were led and completed by excellent postdocs (S. Holalu, J. Vandenbrink, H. Atamian, N. Creux) and technicians (L. Henry and A. Garner). The projects at Morven Farm also involved the critical assistance of many dedicated undergraduates (E. Brown, C. Graham, A. Fortugno, E. Patterson, G. Marino, C. Lu Simon-Plumb, L. Poussard, A. Miller, A. Wofford, S. Toney, S. Krantz, R. Boone, and W. Poehlman). We thank the National Science Foundation Plant Genome Research Program for funding support (IOS-1238040) and the University of Virginia Foundation for use of the plots at Morven Farm. (Liu et al., 2010)

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EVALUATION OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) SINGLE CROSS HYBRIDS UNDER HEAT STRESS CONDITION

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ABSTRACT

Sunflower is an important oilseed crop which shows susceptibility to heat stress. In present study, 63 single cross hybrids were evaluated under heat stress condition for two years and compared with the two commercial hybrids. Genotype and genotype × environment (GGE) was used to differentiate single cross hybrids on the basis of multiple traits. GGE biplot showed that several single cross hybrids had higher seed yield potential than standard checks. Moreover, SYP was related with pollen viability showing that achene yield was product of high gametophytic fertility under heat stress. Hybrids having high seed yield potential under heat stress had lower cell membrane injury (CMI) showing that potential hybrids could be selected on the basis of CMI during seedling stages. GGE biplot for SYP and its components showed that single cross hybrids were characterized into two major groups. Group I was further characterized into two sub group. Group Ia included hybrids with high 100-SW, while group Ib had the hybrids with high number of achenes head^{-1} and head diameter. Group II had the hybrids with high kernel weight and kernel to achene ratio. The hybrids could be recommended according to their potential utilization in the seed industry.

Key words: Achene, gametes, kernel, cell membrane injury, heterosis, biplot

INTRODUCTION

Heat stress is a major production constraint in summer crops which causes significant repressing effects on grain yield and quality (Kalyar et al. 2014; Niazi et al. 2014). Sunflower reproductive cycle has also been subjected to heat stress which reduces the achene and oil yield in various parts of the world (Rondanini et al. 2003; Rondanini et al. 2006; Kalyar et al. 2014; Van der Merwe et al. 2015). High temperature induced fine tuning of canopy architecture such as leaf and petioles angles (Kalyar et al. 2013b). Moreover heat stress reduces the pollen viability, seed germination, leaf area, reproductive biomass and increased cell membrane injuries when sunflower breeding populations were subjected to the reproductive heat stress (Kalyar et al. 2014). It was observed that heat stress accelerate the heat unit accumulation and thus reduce the growth period (Rondanini et al. 2003). The brief exposures of capitula to the heat stress > 35°C decreased the grain weight and oil contents by 40% and 30% respectively due to reduction in growth period (Rondanini et al. 2003). Exposure of temperature greater than 29°C at 10-12 days after anthesis for period of 7 days reduced grain yield by 6% (Rondanini et al. 2006). Corbineau et al. (2002) noted that exposure of the sunflower seed to high temperature of 45°C inhibits its germination and it also increased the electrolyte leakage in the incubation medium. High temperature was also

known to modify oil quality and oleic acid has been shown to increase at the expense of linoleic acid (Flagella et al. 2002; Rondanini et al. 2006).

Sunflower segregating and advanced germplasm has been screened with various selection criteria for the introgression of heat resistance in inbred lines. Kalyar et al. (2013a) selected segregating plants maintaining medium leaf temperature (T_{leaf}) under heat stress. The plants which maintained medium T_{leaf} had high values of leaf gas exchange traits. The usefulness of this trait was also depicted from high heritability, selection gains and its positive relationship with reproductive biomass. Similarly, Kalyar et al. (2013b) identified differences within F_2 population with respect to leaf inclination and upward leaf inclination was found useful to avoid high post noon temperature and cell membrane injury. Differences between pre and post noon leaf temperature (Δ) was useful criteria for selection of heat resistant plants. Δ was also found useful due to presence of high realized heritability and provided sustainability to achene and oil yield under heat stress when plants differing for Δ were compared in F_4 generation (Kalyar 2015). On the basis of these grounds, progenies obtained from the selection of high Δ were advanced to derive 19 CMS lines which were crossed with 5 different restorer lines to develop 63 single cross hybrids. These single cross hybrids were then tested under heat stress condition to select prominent hybrids under prevailing conditions.

MATERIAL AND METHODS

The studies were carried out in the research field of the Department of Plant Breeding & Genetics, University College of Agriculture, Sargodha during the year 2014-15.

DEVELOPMENT OF PLANT MATERIAL

Development of heat stress resistant plant material was started in 2008. Heat resistant breeding material was selected in promising F_2 crosses. Selection was carried out on the basis of plant ability to maintain low post noon temperature as compared to pre noon leaf temperature (Δ). Effectiveness of the selection was further tested in selected progenies on the basis of genetic gain and realized heritability. Promising plants were evaluated for general combining ability. The superior lines were then converted to cytoplasmic lines in back cross scheme. Sixty three single cross hybrids were developed by using 19 cytoplasmic male sterile line and 5 male restorer lines in all possible combinations i.e. each of female line with each of male sterile lines. The lines were selected on the basis of their relative heat tolerance. 63 combinations were obtained from the controlled mating of these lines. In order to obtain cross combinations, male and female lines were planted in experimental field during the crop season February 2013 and 2014. Each line was grown in single row of 4.5 meter containing 14 plants. All female lines floral heads were covered with net bags to avoid pollen contaminations due to insect pollinators. Pollen from each male restorer lines was collected in early morning and dusted over the female line with the help of the camel brush. Pollination was continued until all the stigmas withered on floral head. The developed seed from each cross combination was harvested separately from mature heads, dried and stored in cool place for cultivation in next crop season.

EVALUATION OF PLANT MATERIALS

63 single cross hybrids along with check variety HYSUN-33 and S-278 were evaluated for heat stress tolerance in randomized complete block design with three replications at field research area of University College of Agriculture, University of Sargodha during the cropping season 2014-2015. Sowing was done on raised bed of 75 cm consecutively for two years on March 15, 2014 and March 18, 2015 to expose the hybrids reproductive growth cycle to the high temperature of May-June (Figure 1).

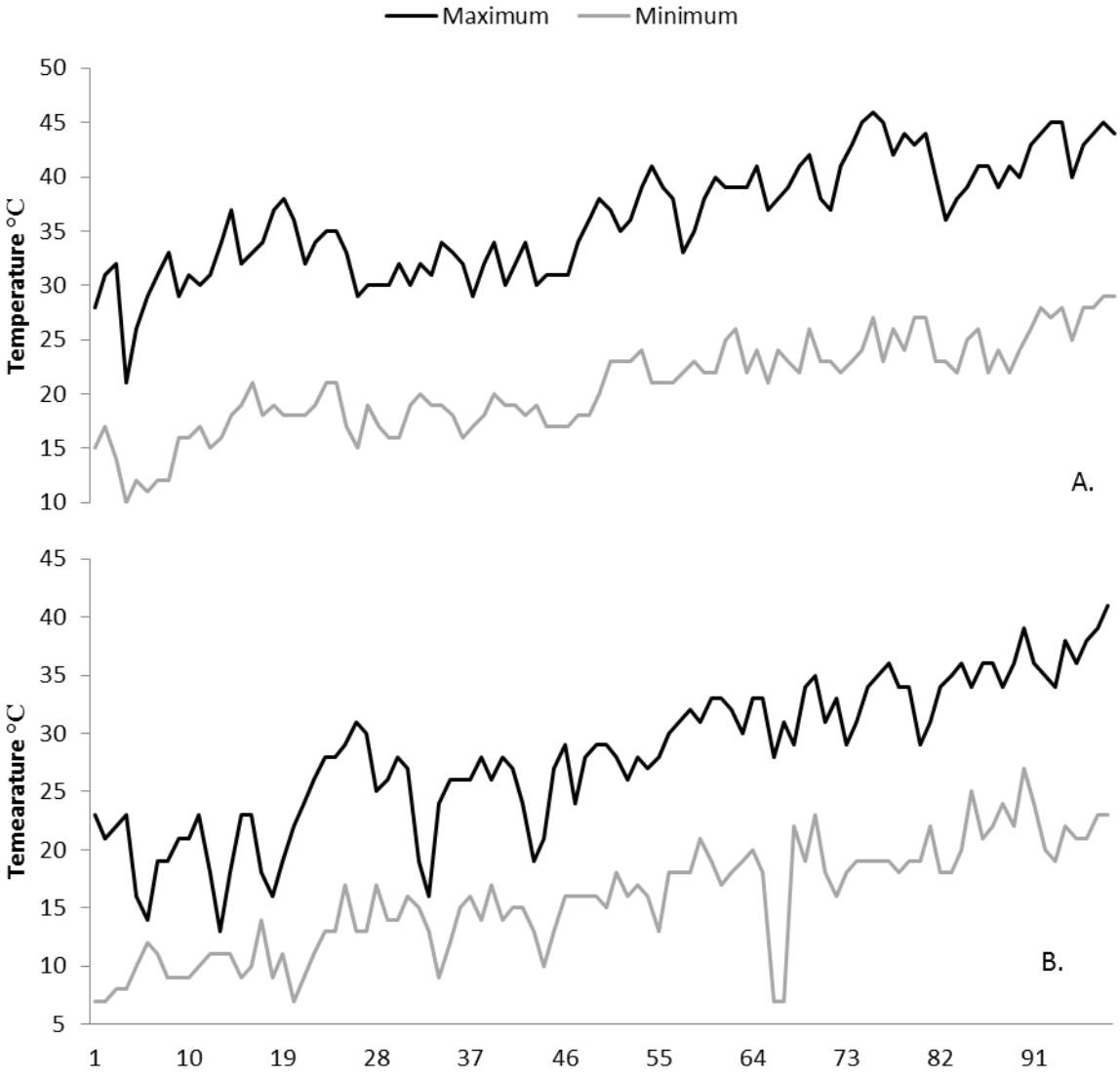

Figure 1. Mean maximum and minimum temperature during the crop season year 2014 and 2015

Soil texture and minerals analysis showed a sandy loam type of soil with EC=2.91±0.19; EC=2.84±0.33; pH=7.16±0.11 pH=7.07±0.14; available potassium was 226±10.34 and 187 ±7.52 and available phosphorous was 17.34±3.69 and 21.58±5.92 during the year 2014-15 at the time of sowing. Each hybrid was sown in three rows each of 4.5 meter with plant to plant distance of 30 cm. The crop was irrigated from canal water to avoid the water stress condition when soil moisture contents were below the field capacity. The field capacity of the soil was 14% by weight measured through gravimetric method. The fertility of

soil was raised by using inorganic urea and diammonium phosphorus fertilizer at the rate of 70 kg acre^{-1} of nitrogen and 45 kg acre^{-1} of phosphorous. Pest scouting was carried out at regular interval and insecticide was sprayed to control the attack of armyworm (200 mL lufenuron, Match ® Syngenta) and red pumpkin beetles (250 / 100 mL dichlorovos, Diptrex) before they exceed threshold level during the year 2014 and 2015 respectively. Herbicide S-metachlor, Dual gold ® Syngenta was used to control pre-emergent weeds after sowing. The crop was evaluated for various plant traits. Pollen viability was measured at the time of anthesis while yield and yield components were determined at the time of maturity.

POLLEN VIABILITY

Pollen viability was estimated by 2% tri-phenyl tetrazolium chloride stain (Prasad et al. 2006). Disc florets were obtained from each head rows during early in the morning (08:00 h) from each plant. Anthers were sequezzed to obtain pollen grains. The sequzed pollen were collected on clean slides. Pollen were stained by adding a drop of stain. The stained pollen with reddish purple color were considered alive due to formation of insoluble red formazan. The reading was noted 30 min 30 min after staining under (10x) light microscope. Pollen viability was noted as the ratio of stained redish pollen to the total pollen.

MORPHOLOGICAL TRAITS

Seed yield was determined by harvesting five heads from each middle row of each hybrid from each block. Heads were manually threshed and seed was dried to uniform moisture content (12%). Dried seed harvested from each head in a row was weight over digital balance to calculate seed yield head^{-1}. Kernel mass was measured by removing the seed coat of 100 seeds. Kernels mass were measured over the analytical balance. Dead seed was determined by carefully examining 100-kernels and all the black kernels or achenes without any kernels were considered as dead. Dead seed (%) was calculated by dividing no. of dead seed to the total no. of seeds observed. Random sample of 100 seeds was counted manually and mass was measured over digital balance. No. of achene head-^{1} was counted through seed counter. Plant height was measured with measuring tape from stem base to the attachment of head. Head diameter was also measured with scale. Oil contents were estimated through petroleum ether extraction on Soxhlet apparatus. Achene size was determined through vernier caliper.

CELL MEMBRANE INJURY

Cell membrane injuries (CMI) were determined through electrolyte leakage in the leaf disc in the department of Agronomy, University of Agriculture, Faisalabad. In order to determine the cell membrane injuries in sunflower single cross hybrids, seedlings were raised in small pots of 15 × 4 cm. Two seeds were sown in each pot which was thinned to single seedling after germination (DAE). First true leaves were tagged on seedling and leaves of similar age (15 DAE) were used to determine the cell membrane injury. Temperature was maintained at 25±2 and humidity was 45%. Photon flux density was 650 µmol m^{-2} s^{-1}. Experiment was laid out in completely randomized design with six replications. Four leaf discs of 5mm in size were dissected from each leaf. Leaf disc were put in glass vial. Two set of leaf disc having three vials for each hybrid was created by treating the leaf disc with two temperature regime. One set was kept at room temperature 25 °C and other set received a

treatment of high temperature 42-50°C for one hour with 2 °C increment after every 10 minutes. 20 mL of deionized water was added to each vial after two temperature treatment. Vials were incubated at 10°C for 12 hours, afterward electric conductivity of both treatments was determined. All vials were autoclaved at 121 °C and final electric conductivity was measured. Cell membrane injury was determined using following formula:

CMI%=(1-(T1/T2))/(1-(C1/C2)) ×100

%injury= (100-CMS)

where T1 and T2 are treatment conductivities before and after autoclaving and C1 and C2 are the respective control conductivities.

BIOMETRICAL PROCEDURES

Data obtained was subjected to the analyses of variance under factorial arrangement where hybrids and years were considered as factors. Biometrical parameters such as genotypic, phenotypic, GCV% and heritability over year were measured as outlined by Allard (1960) where $\sigma^2 g$ (genotypic) = (MSg-MSgl)/rY where MSg is the mean sum of square due to hybrids, MSgl is mean sum of square due to interaction of hybrids × year and r= replication and Y was year). $\sigma^2 p$ (phenotypic)= $\sigma^2 g + \sigma^2 gl + \sigma^2 e$. Heriatbility = ($\sigma^2 g / \sigma^2 p$) x 100 ($\sigma^2 g$ = genotypic variance/σ^2 p=phenotypic variance). Genotypic coefficient of variation (GC%) = ($\sigma^2 g$ /X) × 100. A genotype plus genotype by environment (GGE) analysis (Yan & Kang 2003) was carried out to analyze the heat tolerance traits and yield in order to select promising single cross hybrids under heat stress. Another biplot was developed to study the relationship of yield components and select promising hybrids on the basis of multiple yield contributing traits. The traits were standardized before the analysis in accordance with different scales of the chosen variables. The biplot calculations were made using the 'scale' and 'svd' procedures of the R software (R 2013).

RESULTS

Analysis of variance showed significant variation ($P \leq 0.01$) for single cross hybrids and years for all traits under study. However, interaction due to single cross hybrids and year was significant ($P \leq 0.05$) for traits such as seed yield plant^{-1} (SYP), head diameter (HD), leaf area (LA), dead seed (DS%), kernel weight (KW) and kernel to seed ratio (K to S). Significant ($P \geq 0.05$) interaction showed that single cross hybrids changed their relative ranking for these traits. Hertiability estimates for various yield and its components was moderate to low. Heritability estimates for SYP was moderate which showed that high yielding hybrids may be directly selected through SYP per se under heat stress environment. Traits such as 100-SW, PH, DS%, KW and K to S had moderate heritability while traits such as HD, PV and LA had low heritability (Table 1-2). Among the traits, the highest heritability was shown by SPH. Thus, SPH could also be used along with SYP to determine the yield potential of hybrids under heat stress environment. Among the traits the highest phenotypic variation was shown by leaf area and seed yield while SYP showed the highest genotypic variation among single cross hybrids.

GGE biplot analysis was carried out to characterize single cross hybrids on the basis of multiple traits relevant to SYP and adaptability under heat stress. GGE biplot analysis characterized the germplasm into two groups on the basis of four selected traits (Fig. 2.). Group 1 included the hybrids with high yield and pollen viability while group II included single cross hybrids with high CMI and DS%. The traits such as PV and SYP were close to each other showing positive relationship between the two traits during the year 2015. It was

also concluded that SYP could also be dependent over high PV under heat stress. Therefore, simultaneous selection for PV and SYP could also be practiced. Hybrids such as H-32 and H-21 had high SYP with good PV during the year 2014 (Fig. 2a) while H-35 had high SYP and PV during the year 2015 (Fig. 2b). Hysun-33 had low SYP with high DS% under heat stress. There was also relationship between CMI and DS%. Hybrid H-51, H-48 and H-32 had the highest DS (ratio) while H-7 and H-26 had high CMI and DS during the year 2014 and respectively 2015.

GGE-biplot yield and its components have been shown in Fig. 4a and 4b. GGE-biplot showed that all chosen traits had positive relationship with yield. Traits characterized the single cross hybrids into two groups. The single cross hybrids were grouped on the basis of HD, SYP, SPH and 100-SW in group I. The yield components such as HD, SPH and 100-SW were found close to SYP and had positive relationship with SYP. H-35 showed the highest HD and 100-SW during the year 2014, and thus seed yield was dependent over seed size in this hybrid (Fig. 4a). H-27 and H-29 had high SYP and NSH during the year 2014 while the same hybrid had the highest SYP, NSH, 100-SW and HD during the year 2015 (Fig. 4b). During the year 2015, H-35 was also characterized as high yielder but its yield was not dependent over higher values of morophological traits. Group II included hybrids with high KW% and K to S ratio. Hybrids with high KW and K to S ratio were good for industrial exploitation. Hybrids such as H-58 and H-40 had the highest KW and K to S ratio in both years (Fig. 4a and 4b).

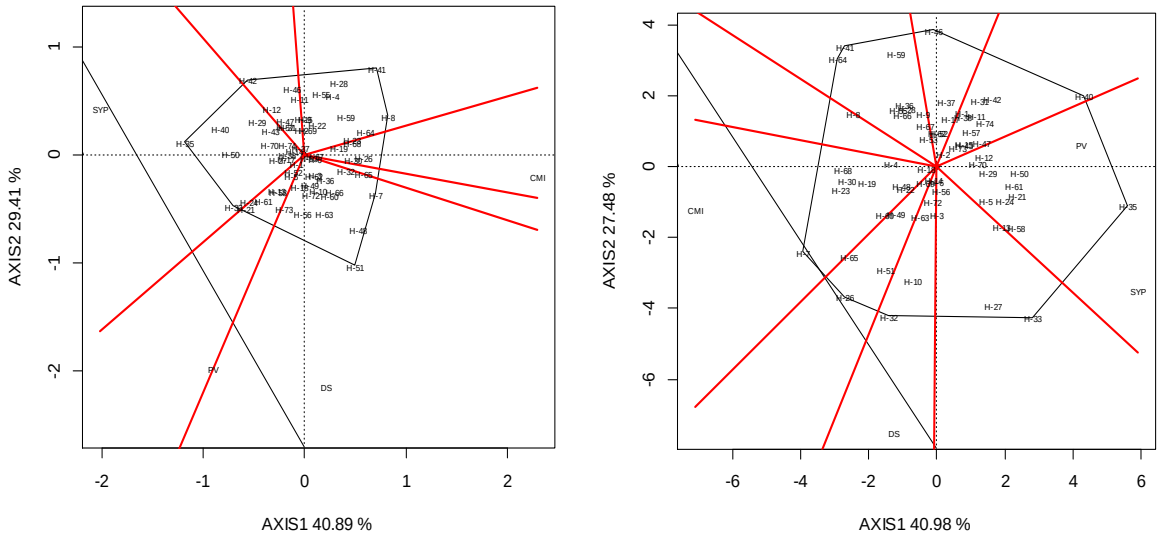

Figure 2. GGE biplot for standardized seed yield plant^{-1}(SYP), pollen viability (PV), cell membrane injury (CMI) and dead seed ratio (DS) for sixty five single cross hybrids under heat stress for year 2014 (a) and 2015 (b). All traits were averaged across replications for each combination of Genotype-by-Trait. The first principal component axis (PC1) retains 49% and the second 29% and 27% of sum of squares for year 2014 and 2015 respectively.

GGE-biplot yield and its components have been shown in Fig. 3a and 3b. GGE-biplot showed that all chosen traits had positive relationship with yield. Traits characterized the single cross hybrids into two groups. The single cross hybrids were grouped on the basis of HD, SYP, SPH and 100-SW in group I. The yield components such as HD, SPH and 100-SW were found close to SYP and had positive relationship with SYP. H-35 showed the highest HD and 100-SW during the year 2014, and thus seed yield was dependent over seed size in

this hybrid (Fig. 4a). H-27 and H-29 had high SYP and NSH during the year 2014 while the same hybrid had the highest SYP, NSH, 100-SW and HD during the year 2015 (Fig. 4b). During the year 2015, H-35 was also characterized as high yielder but its yield was not dependent over higher values of morophological traits. Group II included hybrids with high KW% and K to S ratio. Hybrids with high KW and K to S ratio were good for industrial exploitation. Hybrids such as H-58 and H-40 had the highest KW and K to S ratio in both years (Fig. 3a and 3b).

Mean values of promising hybrids have been shown in Table 1. Results showed that commercial hybrid S-278 had the highest oil contents% followed by the H-58 and H-29. Other commercial hybrid HYSUN-33 had significant lower oil content% than promising hybrid H-58 and H-29. Commercial hybrid S-278 had the lowest oil yield (Table 3). Hybrid H-35 also had higher oil yield than commercial hybrids but had very low oil contents and could be considered as non-oil seed type. On the other hand hybrids H-58 and H-29 could be regarded as oilseed type hybrids for cultivation under heat stress condition. Achene size was estimated on the basis of achene length, width and area. H-35 had the highest while commercial hybrids S-278 had the lowest achene size (Table 1). H-58 had lower achene width than commercial hybrids HYSUN-33.

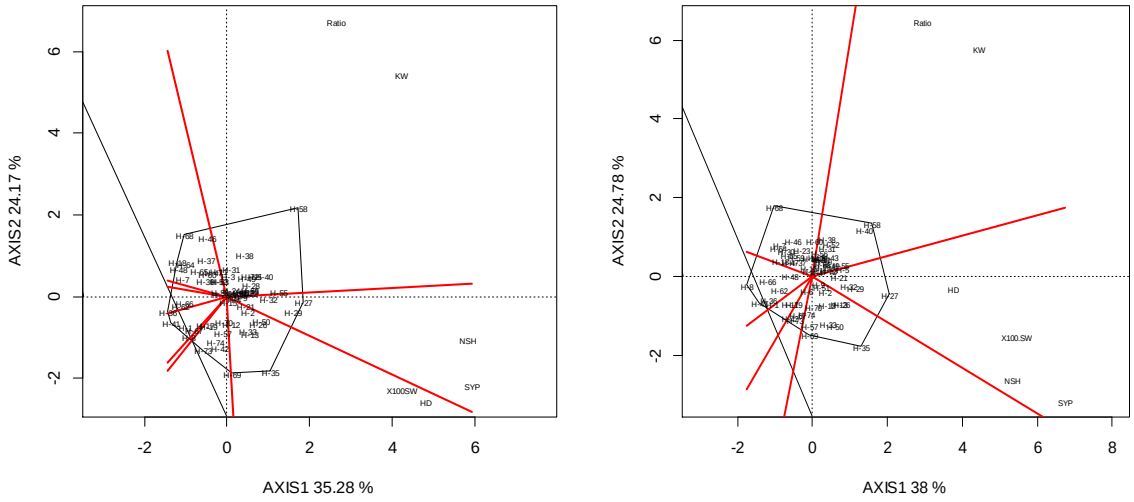

Figure 3. GGE biplot for standardized head diameter (HD), seed head^{-1} (SPH), seed yield (SY) and 100-seed weight (100-SW), kernel weight (KW), kernel to seed ratio (ratio) for sixty five single cross hybrids under heat stress for year 2014 (a) and 2015(b). HD, SPH, SY, KW and ratio were averaged across replications and years for each combination of Genotype-by-Trait. The first principal component axis (PC1) retains 35%, 39% and the second 24% and 25% of sum of squares for year 2014 and 2015 respectively.

Table 1. Variation in mean performance of promising single cross hybrids along with standard hybrids for traits related achene and oil contents.

Hybrid	Year	Oil Content%	Oil Yield (g)	Achene		
				Length	Width	Area
H-58	1	35.32±1.15	27.53±3.47	10.81±0.07	5.27±0.10	56.97±2.38
	2	38.27±2.19	34.83±2.91	9.59±0.05	5.53±0.06	53.03±3.16
	Average	36.79b	31.18a	10.20d	5.21e	53.14d
H-29	1	35.71±2.08	29.85±1.98	11.01±0.05	5.85±0.04	64.41±2.64
	2	36.12±1.52	34.31±2.07	10.52±0.06	5.19±0.08	54.60±3.18
	Average	35.92b	32.08a	10.77c	5.52d	59.42c
H-35	1	24.37±2.65	30.46±3.42	12.85±0.08	7.27±0.05	93.42±1.89
	2	26.48±1.69	35.22±2.77	13.68±0.04	7.85±0.02	107.39±2.38
	Average	25.43d	32.84a	13.27a	7.56a	100.28a
H-27	1	25.29±2.39	29.84±3.41	10.84±0.05	6.48±0.06	70.24±1.93
	2	27.12±1.71	26.04±2.82	11.15±0.06	7.10±0.08	79.17±2.12
	Average	26.21d	27.94b	11.00b	6.79b	74.66b
S-278	1	42.13±1.92	10.95±3.19	9.65±0.08	4.47±0.09	43.14±2.26
	2	39.48±2.19	9.08±2.64	10.52±0.03	5.16±0.06	54.28±2.67
	Average	40.81a	10.02d	10.09e	4.82f	48.56e
Hysun-33	1	32.72±1.09	19.30±1.97	10.70±0.09	5.82±0.08	62.27±3.42
	2	34.16±1.28	19.81±2.14	9.83±0.06	5.63±0.03	55.34±2.34
	Average	33.44c	19.56c	10.27d	5.73c	58.77c

DISCUSSION

Heat stress was one of major production constraints in sunflower which caused significant yield losses of sunflower (Kalyar et al. 2014; Rondanini et al. 2006; Kalyar et al. 2014; Van der Merwe et al. 2015). It was observed that heat stress accelerate the heat unit accumulation and thus reduce the growth period (Rondanini et al. 2003). The brief exposures of capitula to the heat stress > 35°C decreased the grain weight and oil contents by 40% and 30% respectively due to reduction in growth period (Rondanini et al. 2003). Exposure of temperature greater than 29°C at 10-12 days after anthesis for period of 7 days reduced grain yield by 6% (Rondanini et al. 2006). Corbineau et al. (2002) noted that exposure of the sunflower seed to high temperature of 45°C inhibits its germination and it also increased the electrolyte leakage in the incubation medium.

In this study, plant material was originally selected for heat resistance on the basis of (Δ) in the initial segregating generation (Kalyar 2015). Δ was also found useful due to presence of high realized heritability and provided sustainability to achene and oil yield under heat stress when plants differing for Δ were compared in F_4 generation (Kalyar 2015). In comparison to the commercial hybrids, selected progenies showed an advantage of 5%, 47%, 5% and 45% for oil contents (OC%), 100-SW, HD and seedling survival % respectively while 62% and 75% lower unfilled grain% and pollen sterility% over commercial hybrids. Promising progenies were converted to the cytoplasm male sterile lines and mated to the diverse restorer lines to generate single cross hybrids. These hybrids showed substantial genetic variation and moderate heritability for seed yield under heat stress. The variation for seed yield appears due to differences in pollen viability, cell membrane injury and dead

seed%. High yielding hybrids had higher pollen viability showing high seed yield was function of higher gametophytic tolerance (Coast et al. 2015; Das et al. 2014). It has been noticed earlier that heat stress reduces the pollen viability in various species and pollen viability was used as marker to differentiate heat tolerant genotypes (Coast et al. 2015; Das et al. 2014; Kalyar 2015). Hybrids showing high pollen viability were negatively related with cell membrane injury (r^2=-0.43). Therefore, Cell membrane injury could be used to discriminate sunflower hybrids for heat resistance during seedling. Presence of relationship between the pollen viability show that seedling stage heat resistance was also depicted in adult phase heat resistance (Fokar et al. 1998). Lowered cell membrane injuries also tend to reduce the dead seed% (r^2=0.35). High dead seed% affects the oil content to greater extent and thus selection for lower CMI tends to reduce oil yield losses under heat stress (Kalyar et al. 2014).

GGE biplot analysis also partitioned the single crosses hybrids on the basis of various yield components. Generally, GGE biplot partitioned hybrids into two major groups on the basis of yield component. Group I included hybrid on the basis of high 100-SW, seed head^{-1} and head diameter. These traits were significant contributor for seed yield. This group contained two type of hybrids i.e. high seed yield due to higher 100-SW. Thus 100-SW was an important yield component and indicated the importance of greater grain filling or greater seed size for high seed yield. Increased grain filling% has been an important contributor of seed yield under heat stress and indicative of better photosynthates mobilization and food reserve mobilization (Kalyar et al. 2014). However, high 100-SW also tends to reduce seed per head and increase seed size which may reduce the oil yield potential of hybrids. However, our study indicated in-significant ($P \geq 0.05$) relationship between seed per head and 100-SW (r^2=0.15) showing mixed response of hybrids to the increased 100-SW. Another sub group included hybrids with high seed yield head^{-1} and head diameter. Thus photosynthetic process was used to maximize the number of seed per head through high head diameter (Rauf & Sadaqat 2008). Group II included hybrids with high kernel weight and kernel to seed ratio. Both traits are positive contributors to oil yield extraction and thus hybrids with these traits were considered superior for oil yield potential.

It is concluded from the above results that hybrid H-29 and H-58 could be regarded as potential high oil yielding hybrid with good achene yield potential and moderately high heat tolerance. On the other hand, H-35 was promising non-oil seed hybrid with high heat resistance.

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EXPLORING DROUGHT TOLERANCE RELATED TRAITS IN (*HELIANTHUS ARGOPHYLLUS, HELIANTHUS ANNUUS*) AND THEIR HYBRIDS

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ABSTRACT

Drought is major constraint for sunflower (*Helianthus annuus*) production worldwide. Drought tolerance traits have been identified in the related wild species *Helianthus argophyllus*. A study was initiated to develop sunflower drought tolerant germplasm by crossing cultivated sunflower with this species and analyze drought tolerance traits in the interspecific plant material. *H. annuus* and *H. argophyllus* populations, *H.annuus* intraspecific hybrids and *H. annuus* × *H. argophyllus* interspecific hybrids were grown along with the commercial hybrid Hysun-33 under three stress regimes induced by PEG-6000 (T_3) and application of abscisic acid through foliar spraying (T_1) or irrigation (T_2), along with a control (T_0). Morpho physiological traits and growth regulators contents were assessed. Exogenous application of ABA, both by foliar spray and irrigation, had a negative impact on leaf area, stomatal conductance, transpiration and photosynthesis. Across the different groups of germplasm, there was a negative association between leaf area and stomatal conductance and a positive association between excised leaf water content, stomatal conductance and cuticular wax content. *H. argophyllus* populations had a significantly lower leaf area and higher water use efficiency, leaf cuticular wax content under all treatments and maintained higher net photosynthetic rate and stomatal conductance under osmotic stress. Small leaf area and high cuticular waxes content of the wild species were however not inherited in interspecific hybrids, suggesting to rather select transgressive segregants in F2 for these traits. The higher accumulation of indole-acetic acid observed under stress in *H. argophyllus* could contribute for a more developed root system. *H. argophyllus* lines accumulated less zeatin and gibberellic acid and more abscisic acid than the other groups of germplasm, but were able to maintain a higher zeatin content under stress that could result in maintaining growth and biomass. *H. argophyllus* populations and interspecific hybrids showed significant lower accumulation of silicium in all stress environments, reflecting reduced transpiration. These results are discussed with a view of using *H. argophyllus* to improve drought tolerance in cultivated sunflower.

Key words: cuticular wax, growth regulators, interspecific crosses, leaf area, leaf gas exchange parameters, silicium

INTRODUCTION

Drought is a major yield limiting factor for sunflower (*Helianthus annuus* L.) production in semi-arid regions (Ravishankar and others 1991, Rauf 2008, Rauf and others 2015). Oil and achene yield losses due to drought stress were reported in various parts of the

world (Jasinkas 1999; Kazi and others 2002; Hussain and others 2010; Woli and others 2014; Yin and others 2014). Water shortage induces significant modifications in physiological and biochemical processes involved in biomass production and modifies dry matter partitioning (Rauf and Sadaqat 2007; Rauf and Sadaqat 2008a Baloğlu and others 2012; Fernández and others 2012). Breeding for drought tolerance is consequently essential to reduce yield losses in sunflower in drought-prone areas (Adiredjo and others 2014; Rauf and others 2015). In order to identify possible sources of drought tolerance, cultivated sunflower germplasm from diverse sources was evaluated on the basis of relative performance under drought stress (Rauf and Sadaqat 2008b). However, the narrow genetic base of cultivated germplasm has limited the scope of these studies.

Some sunflower wild related species have been reported as drought tolerant and the introgression of traits from these species is expected to increase drought tolerance in the cultivated germplasm (Jan and others 2014). Among the related species, *Helianthus argophyllus* L. was identified as particularly drought tolerant (Jan and others 2014). Based on this information an experiment was carried out to identify possible traits of drought tolerance in *H. annuus*, *H. argophyllus* and their interspecific progenies, by using abscisic acid to induce drought like symptoms and by irrigating plants with a solution of polyethylene glycol (PEG-6000) to create osmotic stress. Genotypic variations were previously observed in sunflower plants submitted to exogenous application of ABA for production of abscisic acid (Ouvrard and others 1996) and maintenance of relative water content and yield (Hussain and others 2010) while the use of PEG has proven to be efficient to screen for tolerance to osmotic stress in this crop (Khalil and others 2015). In the present study, the traits assessed included morphological traits (leaf area, plant height, biomass), physiological characters (net photosynthetic rate and stomatal conductance, excised leaf water loss, epicuticular wax content, leaf silicium content), and plant regulators (gibberellic, indol acetic and abscisic acids and zeatin) contents. These traits are relevant to the drought tolerance breeding and therefore could indicate the type of resistance in sunflower genotypes.

MATERIALS AND METHODS

Plant Material

Three *H. argophyllus* accessions (ARG-1805, ARG-1802 and ARG-1806), introduced from the USDA germplasm collection, and two CMS lines of cultivated sunflower (*H. annuus*) were used in this study. The two lines (CMS-14 and CMS-20) were crossed with *H. argophyllus* accessions to develop six interspecific hybrids. The commercial drought tolerant hybrid Hysun-33 was used as a check. Details of plant material have been shown in Table 1.

Seeds were germinated in large polythene bags containing equal volume of sand, silt and field soil. After thirty days *H. argophyllus* plants, sensible to the photoperiod, were transferred to the field for induction of flowering. At 120 days after emergence, plants were covered from 15:00 to 7:00 during 45 days with black cloth to provide 16 hour dark period. On the other hand, *H. annuus* lines were sown after the start of black cloth treatment to the *H. argophyllus* populations to synchronize flowering. Pollen of the *H. argophyllus* plants was collected at 7:00 in the morning and deposited with the help of a camel brush over the stigma of the CMS lines. Meanwhile, heads of CMS lines were covered with white cloth bags to avoid foreign pollen contamination. Several rounds of pollination were carried out until stigmas completely wither. Interspecific hybrid seeds, as well as seeds of *H. argophyllus* and CMS lines were collected. CMS lines were maintained by tying the floral buds and pollinating the same plants with maintainer lines. Four hybrids were created by crossing *H. annuus* L. female lines CMS-14 and CMS-20 with male fertile lines R-12 and R-18.

Experimental conditions

All the plant material including parental lines and hybrids were evaluated in growth chamber, at the Plant Breeding & Genetics Department, College of Agriculture, University of Sargodha, Pakistan. Two seeds of each genotype were germinated in boxes (10 × 9cm) containing 8.5 kg of field soil: sand: silt. There were three boxes genotypes^{-1}. Temperature was maintained at 25±2°C while humidity was maintained at 40% and light intensity at 650 μmolm^{-2}s^{-1} in all treatments. Treatments included a control (treatment T_0) and three stress treatments. Drought like symptoms were induced through foliar (treatment T_1) and irrigational application (treatment T_2) of 8 μ mol of abscisic acid from germination and at 4 days intervals according to Shinozaki and others (2000), Tuteja (2007) and Fujita and others (2011). Osmotic stress was generated by irrigating plants with a solution containing 50 g L^{-1} of polyethylene glycol (PEG-6000) according to Khalil and others (2015) (treatment T_3).

Plant measurements

Leaf area was assessed using a CI-302 leaf area meter (CID-Bioscience, Camas, USA). Plant height was measured from the base to the top of canopy. Biomass was measured on digital balance. Leaf gas exchange parameters were determined on 26 days old leaves from the top of canopy by using a photosystem CI-340 (CID-Bioscience Camas, USA). Temperature was maintained at 25±2°C while humidity was maintained at 40% and light intensity at 650 μ mol m^{-2}s^{-1} in all treatments. Water use efficiency was ratio of net photosynthesis rate to transpiration (Pn E^{-1})

Excised leaf water loss was assessed according to Dhanda and others (1989), 45 days after emergence, on fully expanded leaves (ie, fifteen days old leaves) at the 2nd node from the top of canopy. Leaves were removed from the plants and their fresh mass was measured immediately on an analytical balance. Leaves were kept at 25°C for six hours to determine wilting leaf mass. Finally leaves were oven dried at 70°C for 24 hours to determine dry mass. Excised leaf water loss was calculated as (fresh leaf mass – wilted leaf mass)/(fresh leaf mass – dry leaf mass).

Epicuticular waxes were determined by the method of Ebercon and others (1977) at 45 days after emergence, on expanded (15 days old) leaves from the top of canopy. Leaf disc of known size (30 cm^2) were dipped in 15 ml pre-distilled chloroform at 25°C for one hour. The extract was filtered, chloroform was evaporated and 5ml of reagent was added to each sample. The reagent was prepared by dissolving 20 g potassium dichromate in 40 ml distilled water. The solution was mixed and further heated for 30 minutes in a concentrated 1 liter H_2SO_4 to make it colorless. Samples were cooled and 12 ml of distilled water was added to each sample. The samples were kept at room and color change was awaited. Optical density (590 nm) of the sample was measured using a spectrophotometer. The standards were prepared by obtaining cuticular waxes from large samples of wild sunflower leaves dipped in chloroform. Obtained waxes (300mg) were mixed in three replicates with the 5ml reagent to each of the replicates and heated until the standard became colorless. The standards were further prepared by mixing stock solution of 0.1 ml, 0.5ml, 1ml, 2ml, 3ml and 5ml to get final concentration of 1μg ml^{-1}, 5 μg ml^{-1}, 10 μg ml^{-1}, 20 μg ml^{-1}, 30 μg ml^{-1} and 50 μg ml^{-1} of standard solution.

Leaf silicium content (μg L^{-1}) was determined according to Dai and others (2005). Leaves samples of each line and crosses were oven dried at 60 °C for at least 7 days. Dried samples were grounded and passed through sieve of 60-mesh. The samples were again dried at 60 °C for 48 hours. 100-mg of sample was poured into polyethylene tubes and 3ml of 50% NaOH was added to each sample. All tubes were covered with loose plastic caps. Tubes were vortex and afterward autoclaved at 121°C for 20 minutes. Volume of plastic tubes was adjusted to

50ml through ddH_2O. 1 ml sample was added to volumetric flask which was further added up by 30 ml of 20% acetic acid and 10 ml of ammonium molybdate (54g L^{-1}, pH 7.0). 5 mL of 20% tartaric acid was added to the tube after 5 min interval followed by 1 ml reducing solution. Volume was adjusted at 50 ml by 20% acetic acid. Measurement was done at 650 nm on spectrophotometer (UV 2600).Standards were prepared by taking 1g ultrapure SiO_2 and slowly heating it to 1000°C in muffle furnace. Temperature was stabilized at 1000°C for 1 hour. 0.1g of treated SiO_2 was further transferred to nickel crucible and slowly heated to 1000°C after adding 2g of Na_2CO_3 to form a lucent melt. Crucible was taken out from the furnace and 5mL of boiling ddH_2O was added in the crucible. The melt was transferred to plastic bottle which was further dissolved by adding 150 mL of ddH_2O. Finally the volume was raised to 1000 ml in volumetric flask and solution was transferred to plastic bottle. Bottle contained stock solution of 0.1 mg mL^{-1} of SiO_2.

Plant growth regulators were determined according to Ergün and others (2002). Each plant sample (leaves) (2g) was grounded in a 60 ml solution (methanol: chloroform: 2N ammonium hydroxide, 12:5:3 v/v/v). The obtained plant extract was treated with 25 ml distilled water. Upper whitish phase was aspirated and chloroform phase discarded. Whitish phase (water-methanol) was further used to evaporate the methanol in extract through rotary evaporator. Obtained water phase was treated with 15 ml ethyl acetate at 2.5, 7 to obtain free form of plant growth regulator. The extract was adjusted at 11 pH and hydrolyzed at 70°C for one hour and plant extraction was done at 2.5, 7 pH to get bound form of plant growth regulators. Ethyl acetate in all plant extract was evaporated at 45°C through rotary evaporator to minimum level to have concentrated sample. The concentrated samples were treated with 1 ml methanol and were run on TLC plates (Silica Gel, 254, Merck Chemicals Germany) to separate gibberellic acid(GA_3), indole-3-acetic acid (IAA) and abscisic acid (ABA) for the samples obtained at pH 2.5 and zeatin at pH 7. Plant growth regulators were extracted through glass plaque with reference to RF value of synthetic plant growth regulators (ABA, zeatin, GA_3 and IAA). Obtained samples were treated with the 1.5 ml methanol and filtered. The samples were then analyzed on spectrophotometer (UV 2600) to determine the optical density along with standards. Optical density was determined at 280 nmfor IAA, 254 nm for GA3, 263 nm for ABA and 269 nm for zeatin.

RESULTS

Plant biomass was reduced 12%, 8% and 34% in the treatments T_1, T_2 and T_3 respectively, compared to the control T_0 (Table 2). Hysun-33 showed the highest plant biomass and *H. argophyllus* the lowest in all treatments. Interspecific crosses had similar plant biomass as Hysun-33 in T_o, T_1 and T_2 but a significantly higher biomass than the commercial hybrid in T_3. Plant height decreased by 26%, 29% and 29% in T_1, T_2 and T_3, compared to T_o (Table 2). Hysun-33 had the highest plant height and *H. argophyllus* the lowest in all treatments. Plant height of interspecific crosses was close to Hysun-33 in T_o and T_1, but significantly lower in T_2 and T_3.Plant height was significantly higher in interspecific hybrids that in intraspecific hybrids in both T_1 and T_2.

Excised leaf water loss (LW) increased with ABA and osmotic stress treatments in all sets of germplasm (Table 3). Averaged over the species and their crosses, the highest LW was noted in ABA foliar treatments. *H. argophyllus* populations maintained the highest LW while interspecific hybrids showed the lowest values in all four treatments. The commercial hybrid Hysun-33 also showed the lowest LW in control treatment. Leaf area decreased by 24%, 11% and 24% T_1, T_2 and T_3, respectively, compared to T_o (Table 3). Hysun-33 had the highest leaf area in this treatment. Leaf area of the commercial hybrid remained unaffected in the three

treatments. Leaf area of interspecific hybrids was similar to this of the commercial hybrid in T_o and T_1, but significantly decreased in $T_2$and T_3.It was higher than this of intraspecific hybrids in T_o and T_1, but not in T_2 and T_3. *H. argophyllus* showed the lowest leaf area in all treatments.

There was a significant decrease in P_N of all genotypes due to stress treatments (Table 4). P_N experienced a decrease of 30%, 57% and 52% in to T_1, T_2 and T_3, respectively. Hysun-33showed the highest P_N in T_o. Net photosynthesis of this hybrid however drastically decreased in T_1, T_2 and T_3. *H. argophyllus* populations along with interspecific hybrids and Hysun-33 showed the highest P_N in T_2. *H. argophyllus* population and interspecific hybrids tend to maintain P_N in all treatments. Interspecific hybrids showed the highest P_N in T_2 and T_3 and *H. argophyllus* populations the highest P_N in T_1. Stomatal conductance was reduced by 71%, 78% and 81% in T_1, T_2 and T_3 treatments (Table 4). In T_0, Hysun-33 showed the highest stomatal conductance and *H. argophyllus* the lowest. *H. argophyllus* had the highest stomatal conductance in T_1 and *H. annuus* in T_2. In T_3, *H. argophyllus* populations and interspecific hybrids showed the highest stomatal conductance.

There was decrease of 58%, 69% and 62% in transpiration rate (E) due to treatments i.e. T_1, T_2 and T_3 (Table 5). Hysun-33 showed the highest E in T_0 and T_1 while crosses (both types) in T_2 and interspecific crosses in T_3. Water use efficiency (WUE) increased by 13%, 17% and 13% in T_1, T_2 and T_3 (Table 5). *H. argophyllus* showed the highest WUE in all treatments.

Silicium content increased by 124%, 90% and 96% in T_1, T_2 and T_3, respectively, compared to T_0 (Table 6). Hysun-33 had the highest silicium content in T_o, T_2 and T_3. Silicium content was similar in the commercial hybrid and intraspecific hybrids inT_2. *H. argophyllus* and interspecific crosses showed the lowest silicium content in all treatments. Leaf cuticular waxes decreased as a result of the ABA treatments and osmotic stress. There was decline of 46%, 53% and 51% in T_1, T_2 and T_3 respectively when compared to T_o (Table 6). *H. argophyllus* showed the highest leaf cuticular waxes in all treatments. Interspecific hybrids showed significantly lower leaf cuticular waxes than *H.argophyllus*.Hysun-33 showed the lowest cuticular waxes content in all treatments.

Abscisic acid (ABA) content increased by 67%, 133% and 47% in T_1, T_2 and T_3, compared to T_o (Table 7). The highest ABA content was noted in *H.argophyllus* in T_1 and $T_3$and in Hysun-33 in T_2. Interspecific hybrids showed higher ABA contents than intraspecific hybrids in T_2.GA_3 content decreased by 33%, 31% and 15% in T_1, T_2 and T_3 (Table 7). The interspecific hybrids had the highest GA3 contents in T_o and Hysun-33 in T_1, T_2 and T_3. GA_3 content increased under stress in Hysun-33 and decreased in the other groups of germplam. *H. argophyllus* showed the lowest GA3 content in all treatments.

Zeatin content decreased by 12%, 28% and 26% in T_1, T_2 and T_3, compared to T_o (Table 8). Hysun-33 showed the highest zeatin content in all treatments. In T_1 and T_2, *H. argophyllus* and interspecific hybrids had higher zeatin content than *H. annuus* and the intraspecific hybrids. IAA increased by 28%, 17% and 19% in T_1, T_2 and T_3 (Table 8). In all these treatments, *H. argophyllus* and interspecific hybrids had a higher IAA content than *H. annuus* and intraspecific hybrids, respectively. Hysun-33 showed the lowest IAA in all treatments.

In the GGE biplot analysis carried out on physiological traits, the two first components depicted 90.65% of the variation (Fig. 1). In T2, cuticular waxes content was positively related with stomatal conductance. In T_3 stomatal conductance was negatively related with leaf area. In both T_1 and T_2, excised leaf water content was positively related to stomatal conductance. In the GGE biplot analysis carried out on plant morphological traits and growth

regulators, the two first components depicted 71.65% of the variation (Fig. 2). Plant biomass was positively related with zeatin contents in all treatments. ABA content was negatively related with plant biomass in T_1 and T_3, stomatal conductance in T_0 and T_2, zeatin contents in T_3, and net photosynthetic rate in T_1.

CONCLUSION

The present study confirmed the value of *H. argophyllus* to improve drought tolerance in cultivated sunflower, previously reported by different authors. Additional traits of potential interest were detected in this species, like smaller leaves with higher cuticular wax content (which allow reducing evapo-transpiration losses) and capacity to maintain net photosynthetic rate and accumulate more ABA, IAA and zeatin under osmotic stress. Some of these traits, as smaller leaves have to be considered carefully as they could be counter-productive under mild stress or optimal conditions. On the other hand, the effects of growth regulators on final yield under different environmental conditions are not yet fully elucidated. Finally, the inheritance of those traits has to be further investigated. The high excised leaf water content and leaf cuticular wax content under stress of the wild species was not maintained in the interspecific hybrids.

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EFFECTS OF HERBICIDE AND SALINITY STRESSES ON SOME DEFENSE RESPONSES OF SUNFLOWER PLANT

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ABSTRACT

Plants in nature are usually simultaneously subject to various abiotic stress factors such as temperature, drought, salinity and pesticides. Pendimethalin is a herbicide used commonly for fight against weeds with narrow and broad leaves. This herbicide is used commonly in our country especially in areas where cotton, sunflower and vegetables are grown. In this study, defense responses of sunflower plant subjected both separately and simultaneously to herbicide and salinity stresses are investigated. It was found that application of these two stress factors both separately and simultaneously on sunflower leaves caused changes in pigment content, lipid peroxidation level and antioxidant enzyme activities.

Key Words : Sunflower, pigment, lipid peroxidation, antioxidant

IMPACT OF EXOGENOUSLY APPLIED GLYCINE BETAINE ON PHYSIOLOGICAL ATTRIBUTES OF SUNFLOWER UNDER DROUGHT STRESS

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ABSTRACT

Scarcity of water causes physiological, biochemical & oxidative damages in sunflower (*Helianthus annuus* L). Effect of exogenous glycine betaine (GB) application through foliar and Hoagland's nutrient solution in amelioration of water stress of sunflower hybrids was studied. Appropriate time, mode and doze of GB application were tested. Three levels of irrigation were used normal irrigation, no irrigation at vegetative and reproductive stages. Three levels of glycine betaine i.e. 0, 75 mM and 125 mM were applied by means of foliar application at vegetative and flowering stage. Morphological parameters such as root and shoot length, fresh and dry weights were recorded three weeks after the GB application. Physiological and biochemical parameters including leaf water and osmotic potential, turgor potential, relative water content, photosynthetic rate, chlorophyll content, protein, soluble sugars and amino acid content were recorded. Nevertheless exogenous GB improved these attributes. However, the irrigated mode of exogenous GB application was more effective than foliar application, among the doses applied 125mM proved more effective. Role of exogenously applied glycine betaine was more pronounced at flowering stage than vegetative stage.

Keywords: Drought, Glycine betaine, Sunflower, Exogenous.

INTRODUCTION

Water stress is being considered the primary factor in limiting crop production (Ashraf and Harris, 2013) and affect plant growth and productivity (Chaves *et al*., 2009). Glycinebetaine (GB) is an amino acid derivative and many scientists hypothesized that GB in low concentration can improve stress tolerance. GB reduces lipid peroxidation of the cell membranes and prevents deterioration of photosynthetic protein complexes (Holmstrom *et al*., 2000; Iqbal and Ashraf, 2008). Endogenous glycinebetaine concentrations show variation in plants, with some taxa accumulating the compound naturally, whiles not others (Bluden *et al*., 2001).

Sunflower (*Helianthus annuus*) is important oil seed crop and its yield is adversely affected by water stress. Water stress affects sunflower plant growth and biomass production (Tahir and Mehdi, 2001). Water stress at vegetative and reproductive growth phase in sunflower may result in 61% and 40% yield reduction, respectively (Bluden *et al*., 2001; Iqbal, 2004). Osmolytes production in water stress condition is an important physiological adaptation to minimize the detrimental effect of stress and achieved by the accumulation of osmolytes such as proline and glycinebetaine (Holmstrom *et al*., 2000). GB accumulation in plants helps to reduce adverse effect of water stress (Iqbal, 2004; Yang and Lu, 2005). Exogenous GB may have also enhanced the ability of cells to retain water without disturbing the cellular functions. Limited knowledge is available about, effective concentrations of GB,

timings and frequency of exogenous GB application. This is prerequisite for GB role in crop stress tolerance. Therefore, the present study is a step towards determining appropriate doze, mode and time of application of GB that could be more beneficial to alleviate the water deficit condition.

MATERIALS AND METHODS

This study was carried out at glass house of department of botany PMAS Arid Agriculture University, Rawalpindi. Seeds of both hybrids (hybrids Hyoleic-41 and Hysun-33) were brought from crop science, NARC Islamabad. Sodium hypochlorite 5% solution was used for surface sterilization. Uniform size seedlings were transplanted to earthen pots containing 10 kg soil and having 40 cm diameter. Soil used: compost, sand, farmyard in a ratio of 2:1:1. Drought stress was imposed by obstructing the water for 9 days at two stages of plant that is vegetative and reproductive stages. Three treatments of glycine betaine were applied with the onset of drought stress at both the stages of sunflower hybrids. Two differnet mode of Glycine betain application were applied wiz irrigation of plant with GB dissolved in Hogland nutrient medium; and through foliar application by hogland solution subsequently.

Morphological Attributes:

Total number of leaves per plant were counted for vegetative growth features after application of GB for three weeks. Root and shoot fresh weight was determined after harvesting. For dry weight of plants the root and shoots were dried at 65 ^{0}C in oven.

Physiological Attributes:

For the determination of relative water content, Unyayar *et al*. (2005) method was adopted. Leaf water potential was calculated by Scholander pressure chamber (Scholander *et al*., 1965). Freezing point osmometer was used for calculation of osmotic potential of flag leaf, turgor pressure was calculated as well (Garnier and Berger, 1985). For each treatment photosynthetic rate was measured using a portable photosynthesis system (Infrared Gas Analyzer. ADC-LCA-4). Leaf chlorophyll content was analyzed by method of Hiscox and Israelstam (1976)

Biochemical Attributes:

A sample extract was used to determine spectrophotometrically by Bradford method for the determination of protein (1976), Ninhydrin method was used for the determination of amino acids in flag leaf extarct (Hamilton and Van Slyke, 1943). Soluble sugars were estimated by Dubois *et al*. (1951) method. Bates *et al*. (1973) method was used to calculate proline content of plants.

Statistical Analysis

Data were statistically analyzed using Statistics 8.1 program by comparing means by LSD at significane level $P \leq 0.05$.

RESULTS

Morphological Parameters

Water stress is one of major abiotic stresses that drastically reduce plant growth and productivity. Water stress decreased shoot fresh and dry weight at both vegetative and reproductive stages. When shoot fresh weight data was subjected to ANOVA ($p \leq 0.05$) significant difference was observed. In stress conditions shoot fresh weight was comparatively higher at reproductive stage 125 mM Gb via foliar application as compared to irrigation GB application (Fig. 1).Among the two varieties v2 (Hyoleic-41) grew better under

stress condition. Hyoleic-41 was responeded more efficiently as compared to Hysun-33 under water stress condition Results revealed that maximum value of shoot fresh weight was observe at T8 and minimum value was observed at T3. Shoot fresh weight of plants with foliar 125 mM GB application was highest with increase of 27% as compared to other GB concentrations. Shoot dry weight when subjected to ANOVA ($p \leq 0.05$) showed considerable difference was observed among all treatments. The most effective treatment was 125 mM GB foliar application at reproductive stage (Fig. 2).GB foliar application at reproductive stage show 29% increase in shoot dry weight and 25% increase due to GB application via irrigation at reproductive stage. The highest shoot dry weight was recorded at T8 (foliar 125 mM Gb application @ reproductive stage) Sunflower shoot and root fresh and dry weight considerably decline under water stress condition. For root fresh and dry weight of sunflower plant under water stress condition subjected to ANOVA ($p \leq 0.05$) a considerable difference was recorded among all treatments. Results of root fresh weight depict that highest root fresh weight was observed at 75 mM GB applied through irrigation at reproductive stage (Fig. 3 & 4). The most effective treatment was T11 for root fresh and dry weight followed by T10 (125 mM GB applied via irrigation at vegetative stage). In root fresh and dry weight GB applied via irrigation was more effective as compared to foliar GB application. Root and shoot fresh weight was considerably increased in water stress condition due to GB application. GB act as growth regulator and enhance root length under stress condition. Root length of Hysun-33 and Hyoleic41 was expressively increased at T8 (foliar applied 125 mM GB @ reproductive stage) (Fig. 5). Root length was recorded at T8 and T 12 with an increase of 13% and 12%. ANOVA analysis of number of leaves data showed significant difference among treatments and cultivars. Gb application via foliar or irrigation both methods were effective for number of leaves. Highest number of leaves were observed at T12 with an increase of 46% followed by T8 with an increase of 35% (Fig 6). Shoot growth is severely affected due to water shortage and shoot length was drastically reduced in water stress condition. Statistical analysis of shoot length data was showing significant difference at ($p \leq 0.05$). GB application via foliar spray was seems to more effective in case of shoot length as compared to GB application through irrigation (Fig 7). Hyoleic41 was observed with maximum shoot length as compared to Hysun-33. Shoot length maximum value at T7 & T8 with an increase of 27% and 22%, respectively.

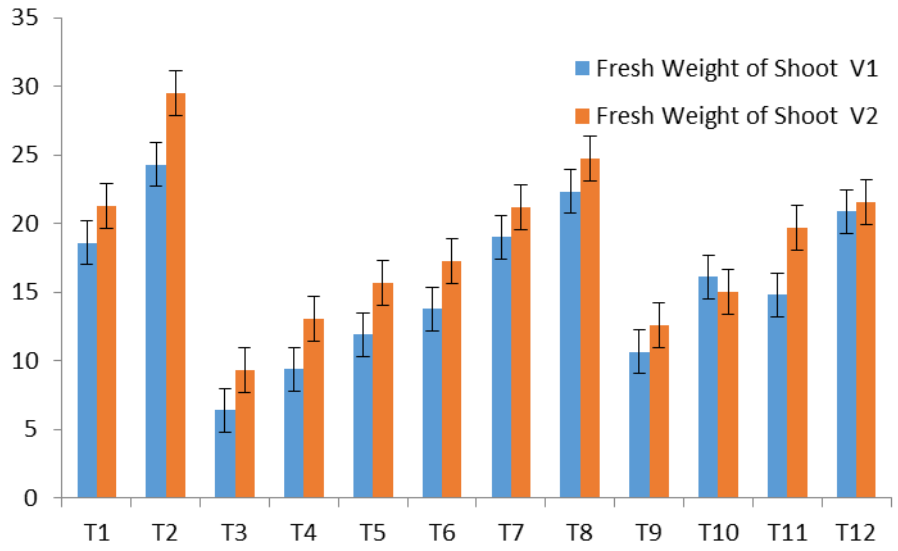

Figure 1: Impact of GB application on shoot fresh weight (gm) of sunflower

T1=Normal irrigation(vegetative), T2= Normal irrigation(reproductive), T3 = stress at vegetative stage, T4= Stress at reproductive stage, T5= Foliar GB application @75mM at vegetative stage, t6= Foliar GB application GB @125mM at vegetative stage, T7= Foliar GB application GB @75mM at reproductive stage, T8= Foliar GB application GB @125mM at reproductive stage, T9= Irrigation of GB @75mM at vegetative stage T10= Irrigation of GB @125mM at vegetative stage, T11= Irrigation of GB @75mM at reproductive stage, T12= Irrigation of GB @125mM at reproductive stage.

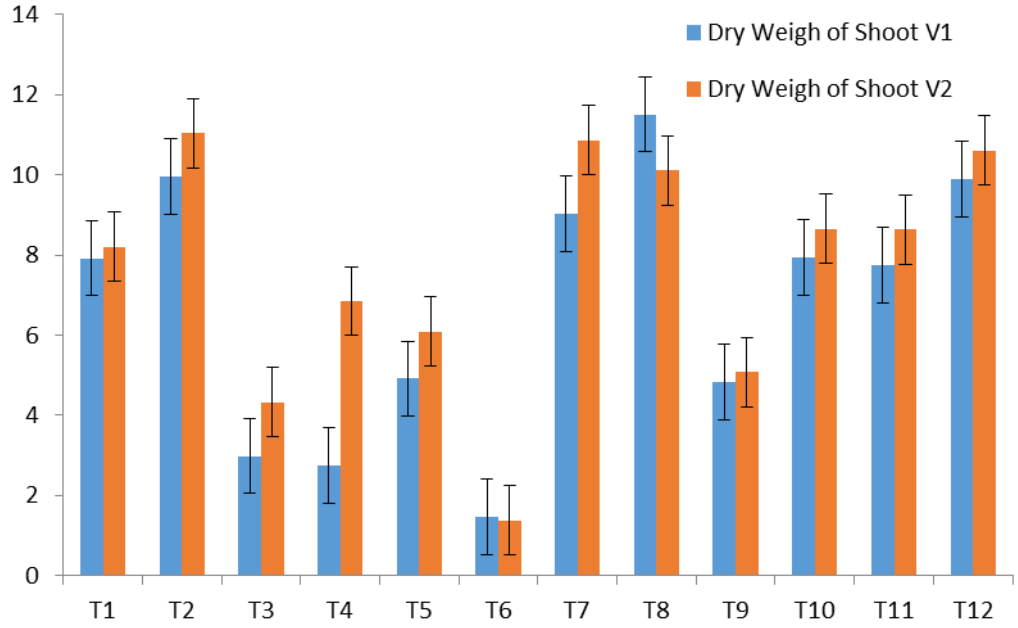

Figure 2: Impact of GB application on shoot dry weight (gm) of sunflower

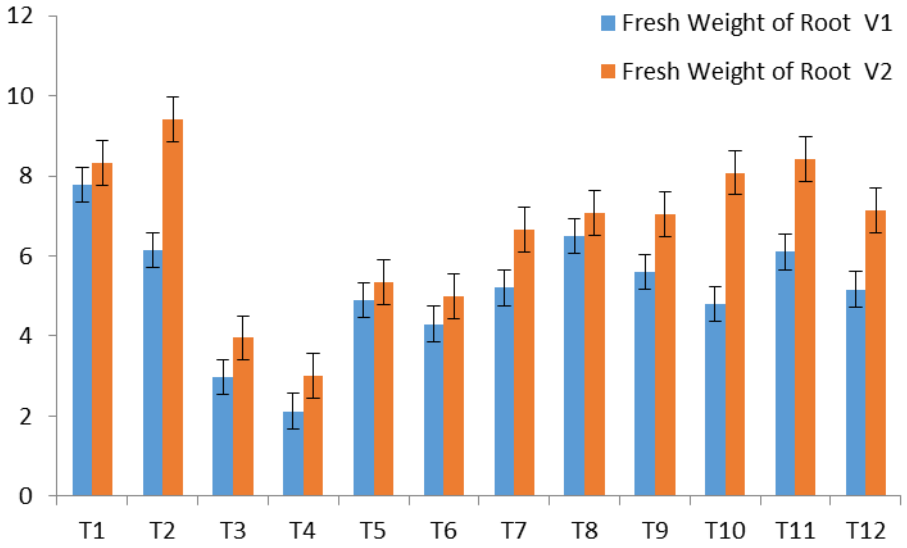

Figure 3: Impact of GB application on root fresh weight (gm) of sunflower

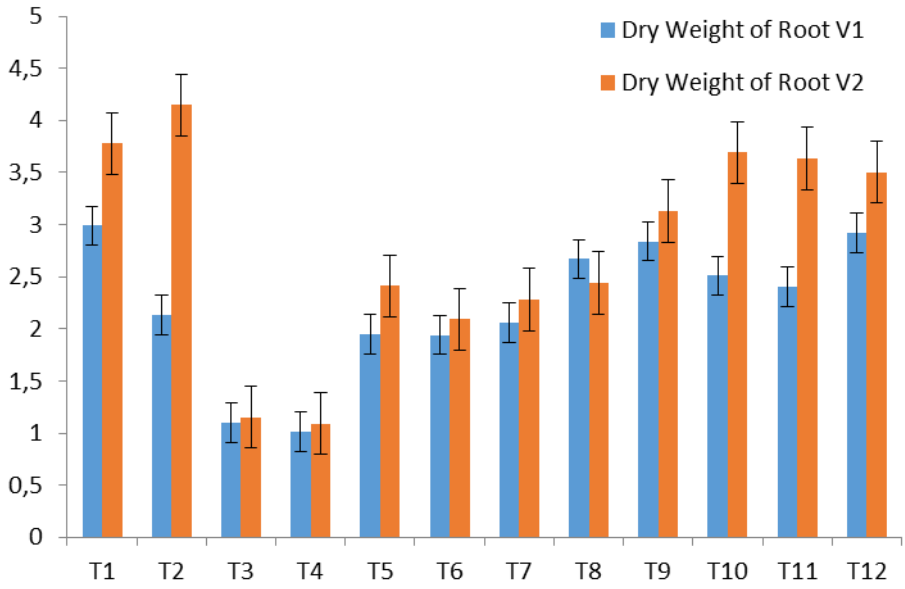

Figure 4: Impact of GB application on root dry weight (gm) of sunflower

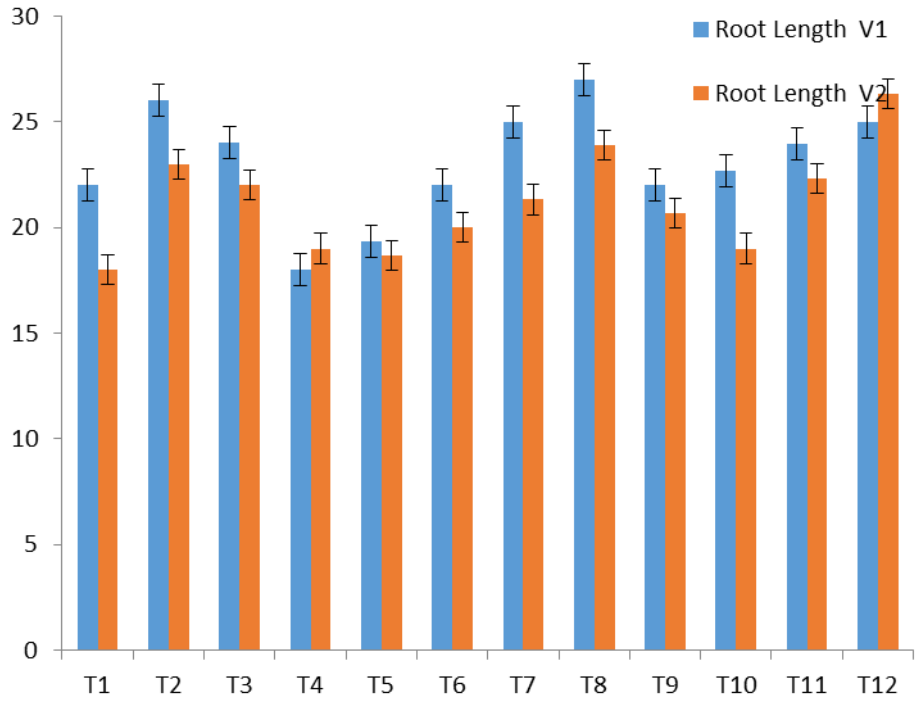

Figure 5: Impact of GB application on root length (cm) of sunflower

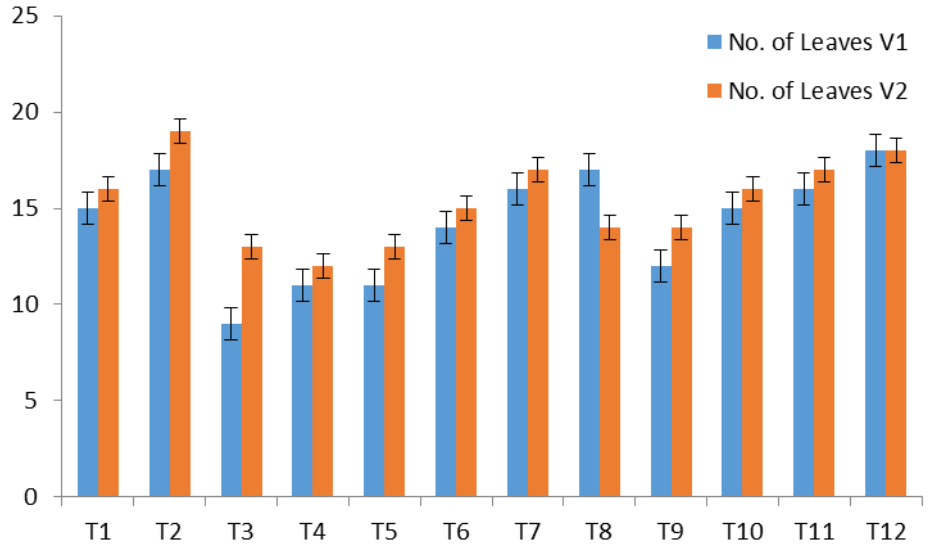

Figure 6: Impact of GB application on number of leaves of sunflower

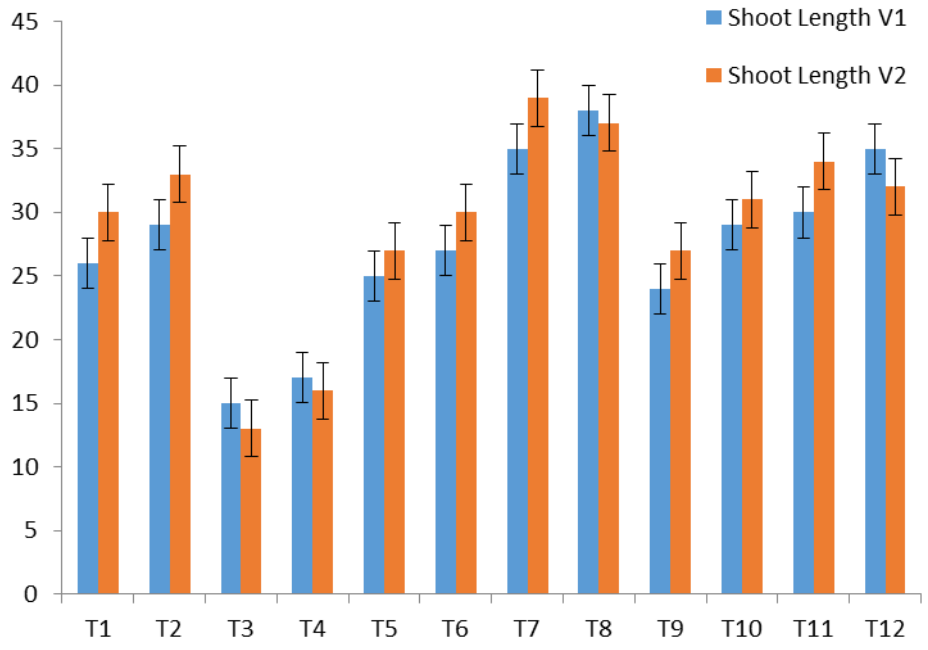

Figure 7: Impact of GB application on shoot length (cm) of sunflower

Physiological Parameters

Drought condition has prominent effect on physiological processes of plant and drastically effect photosynthetic machinery of plant. A considerable decline in total chlorophyll content of plant was observed under water stress condition as compared to control condition. Total chlorophyll content data were subjected to ANOVA ($p \leq 0.05$) and significant difference was detected among all treatments and sunflower cultivars (Fig 8). The most effective treatment for total chlorophyll content in foliar application was T6 (125 mM GB application @vegetative stage) and T8 (125 mM GB application @vegetative stage). Total chlorophyll content showed an increase of 15% and 11% due to GB application via

foliar and irrigation as compared to control condition. Chlorophyll a and chlorophyll b show similar trend (Fig 9 & 10). Treatment effects of chlorophyll a (foliar & irrigated application of 125 mM GB) were more significant at reproductive stage with an increase of 10 and 8%, respectively. Chlorophyll b was negatively influenced by water stress and effective treatment for chlorophyll b was T8 with an increase of 15%. Rate of photosynthesis also had a pattern analogous to transpiration rate in normally watered plants. Photosynthetic rate data were subjected to ANOVA ($p \leq 0.05$) and obvious difference was recorded. GB application via foliar spray was effective for photosynthesis rate (Fig 11). Photosynthetic rate were increased 15% because of GB application as compared to respective control. The present study findings confirmed that GB application at vegetative and reproductive stage are effective to enhance photosynthesis. Plant water relation drastically influenced by water stress. Relative water content results were subjected to ANOVA ($p \leq 0.05$) and showed considerable difference. Relative water content of GB treated plants show an increase of 19% as compared to respective control (Fig 12). The most effective treatment was foliar GB application at reproductive stage with an increase of 17%. Results of water potential were subjected to ANOVA ($p \leq 0.05$) and noteworthy difference was observed (Fig 13). In drought condition without GB application 30% reduction in water potential was observed. The most effective treatments for water potential T6 (foliar 125 mM Gb applied @vegitative stage) and T10 (125 mM Gb applied via irrigation @ vegetative stage). Highest values of water potential were observed in plants treated with GB foliar spray. ANOVA analysis of osmotic potential results showed significant difference at ($p \leq 0.05$) (Fig 14). Osmotic potential of plant without GB treatment under water stress with an increase of 17 and 20 % at vegetative and reproductive stages, respectively. Turgor potential play an important role to maintain turgidity of cell. Turgor potential was recorded and a considerable difference was found (Fig 15).

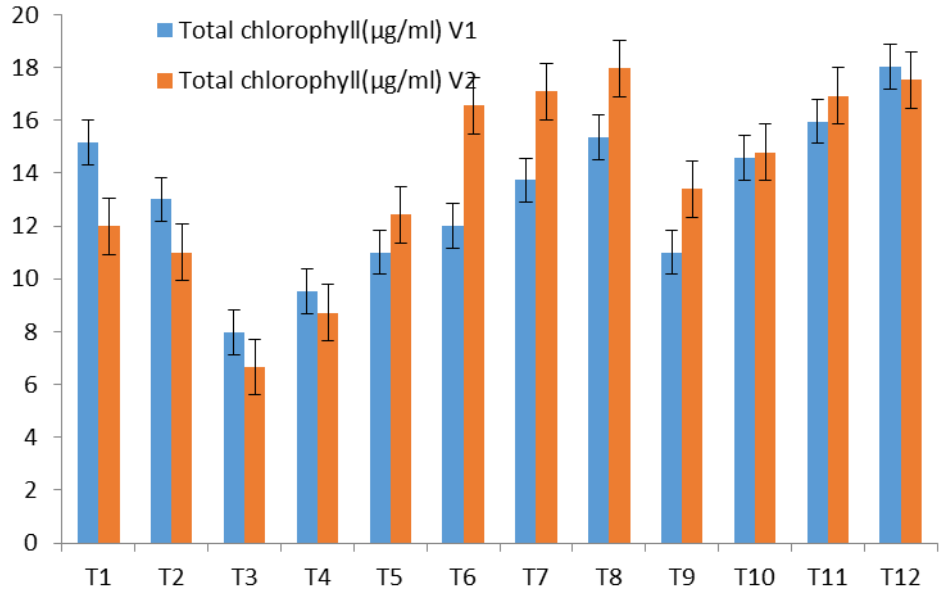

Figure 8: Impact of GB application on total chlorophyll of sunflower

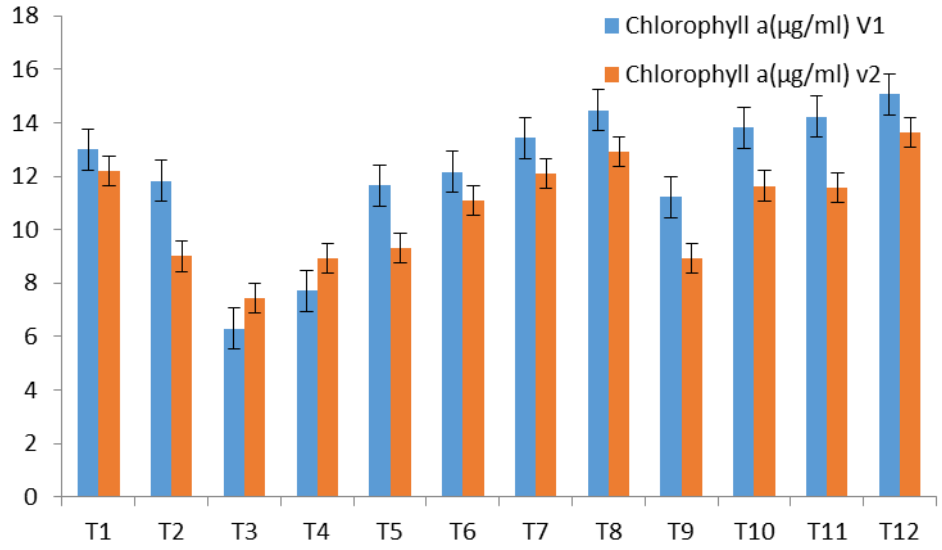

Figure 9: Impact of GB application on chlorophyll a of sunflower

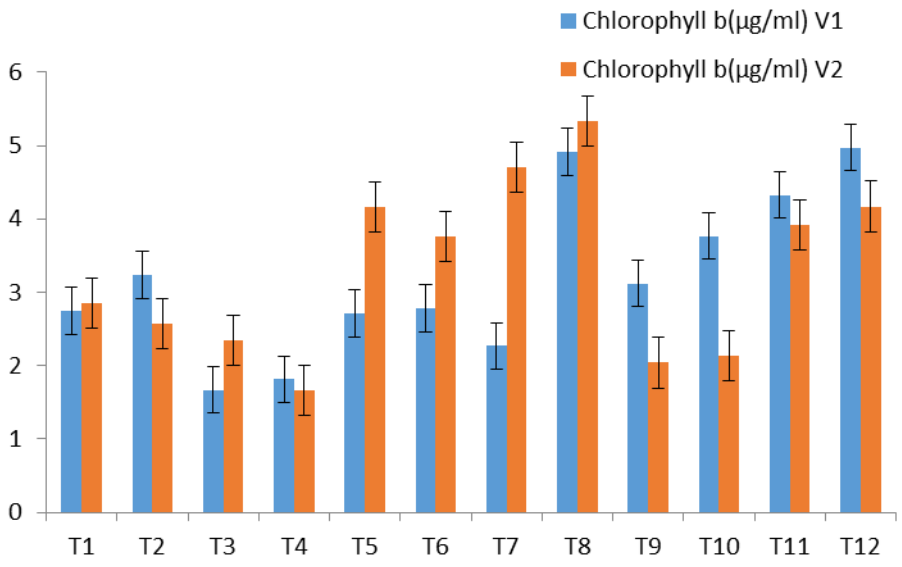

Figure 10: Impact of GB application on chlorophyll b of sunflower

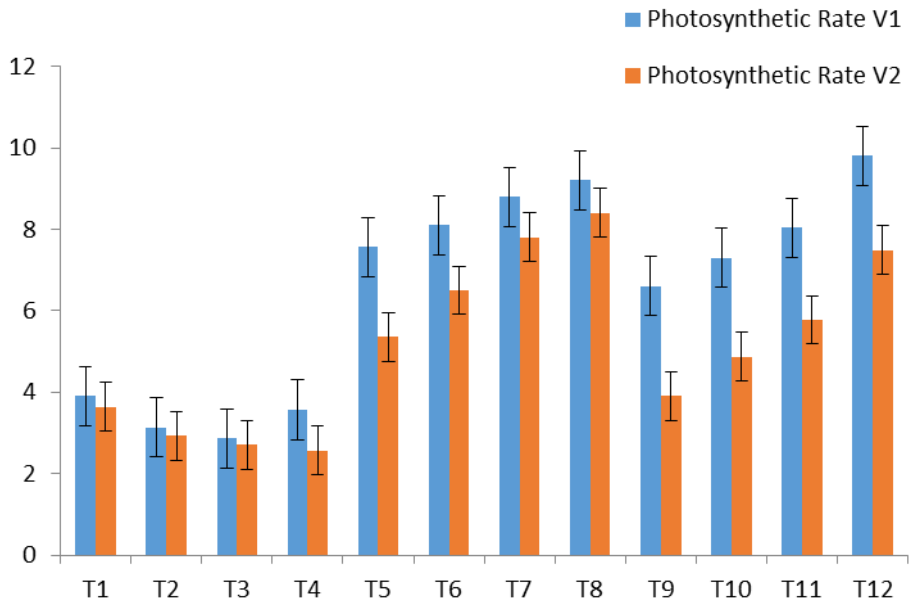

Figure 11: Impact of GB application on Photosynthetic rate of sunflower

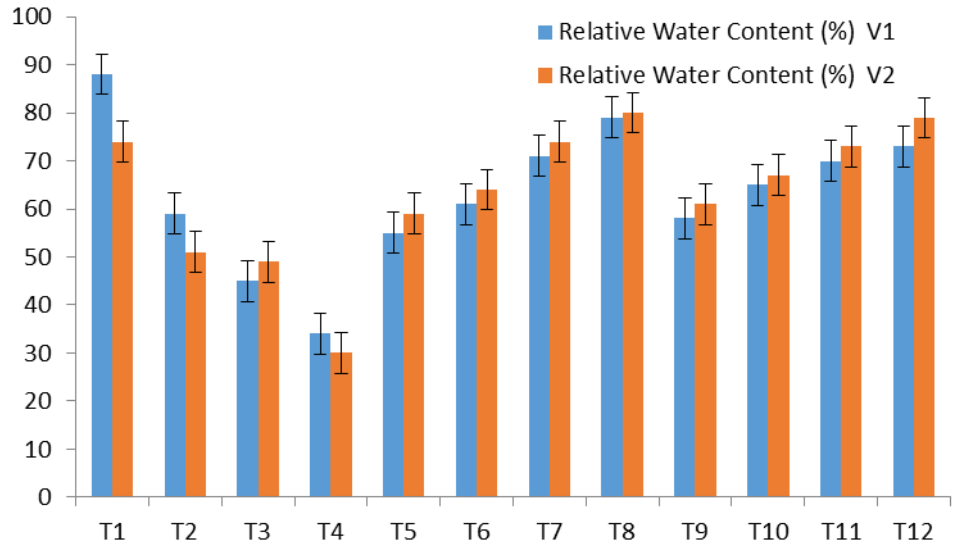

Figure 12: Impact of GB application on relative water content of sunflower

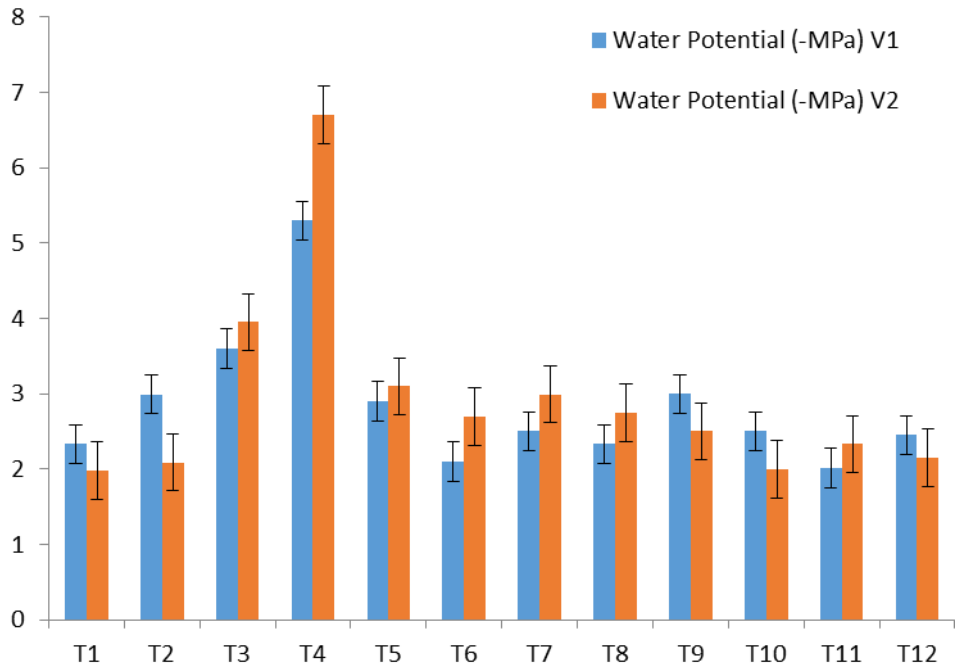

Figure 13: Impact of GB application on Water potential of sunflower

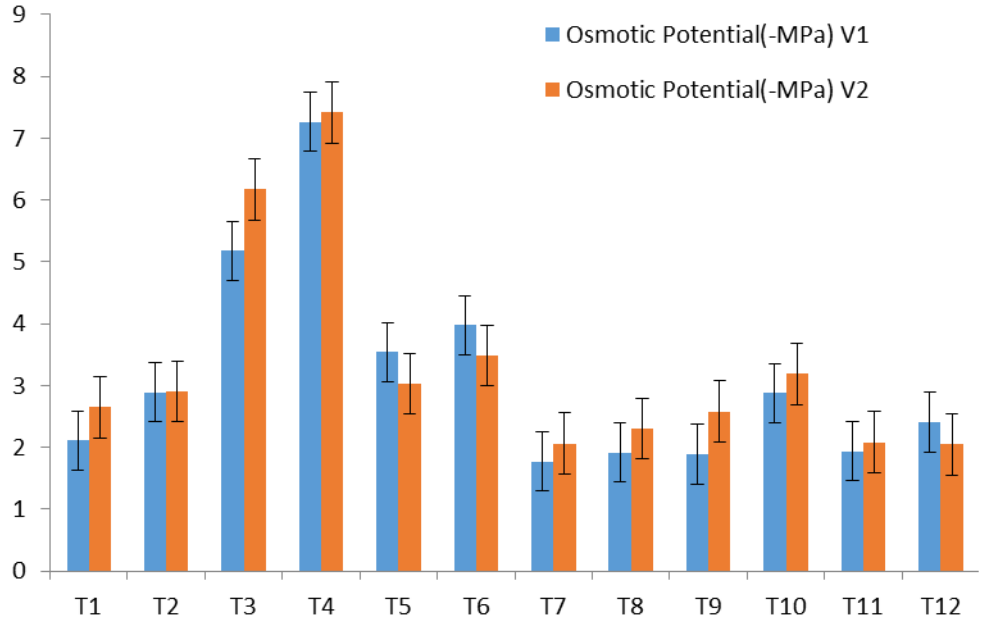

Figure 14: Impact of GB application on osmotic potential of sunflower

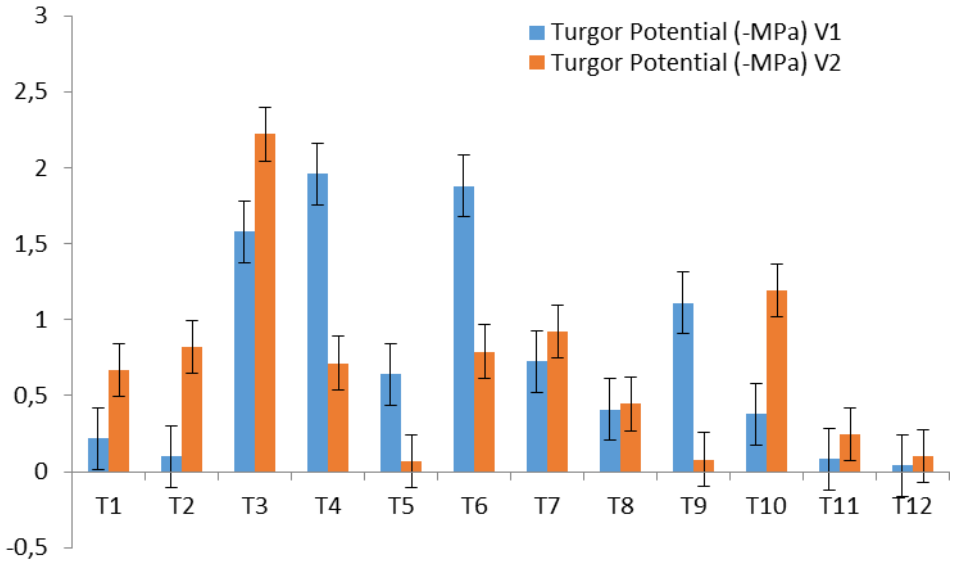

Figure 15: Impact of GB application on Turgor potential of sunflower

Biochemical Parameter

When plants are exposed to abiotic stress condition plant produces different types of osmo protectants that helps plants to tolerate unfavorable condition. Results of protein content were showing significant difference when subjected to ANOVA ($p \leq 0.05$). An increase of 13% of protein content in plants was recorded under water stress (Fig 16). The most effective treatment for GB treated plants were T6 (foliar 125 mM GB application at vegetative stage) and T7 (foliar 125 mM GB application at productive stage). In drought stress condition, it was observed that 21 % increase in free amino acid due to GB treatment via irrigation (Fig 17). The most effective Gb concentration for free amino acid was 125 mM at reproductive stage. Similarly higher content of proline content was observed in water stress condition. Results of proline were subjected to ANOVA and significant difference was observed among treatments (Fig 18). Maximum proline production was observed in GB treated plants with an increase of 23% as compared to respective control. High amount of total soluble sugar was found in water stress (Fig 19). Because in less amount of water, soluble sugar accumulate to combat stress condition. Under water stress without GB treatment an increase of 14% soluble sugar content was observed. Results of soluble sugar content was show significant difference among treatments ($p \leq 0.05$).

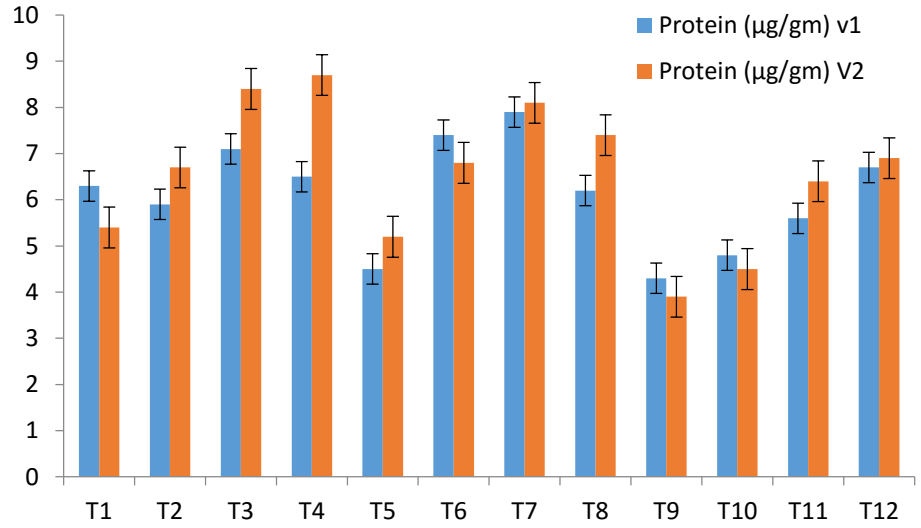

Figure 16: Impact of GB application on protein content of sunflower

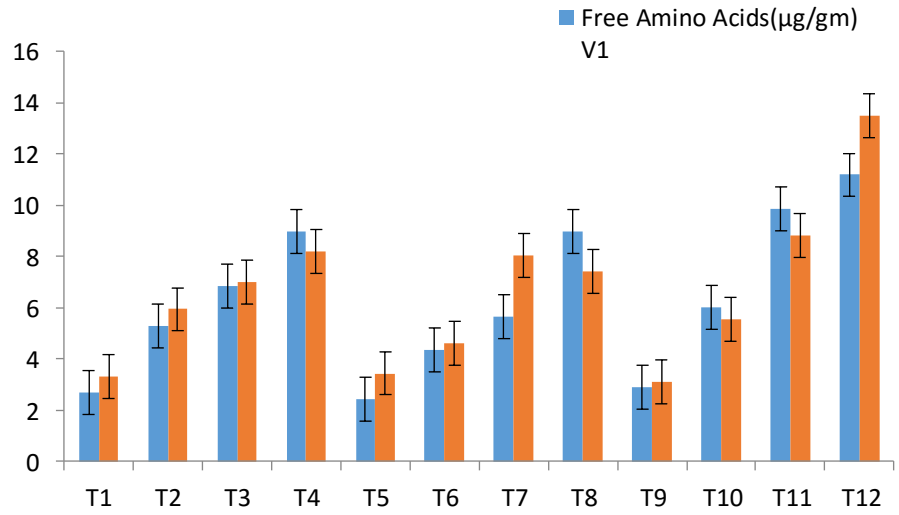

Figure 17: Impact of GB application on free amino acids of sunflower

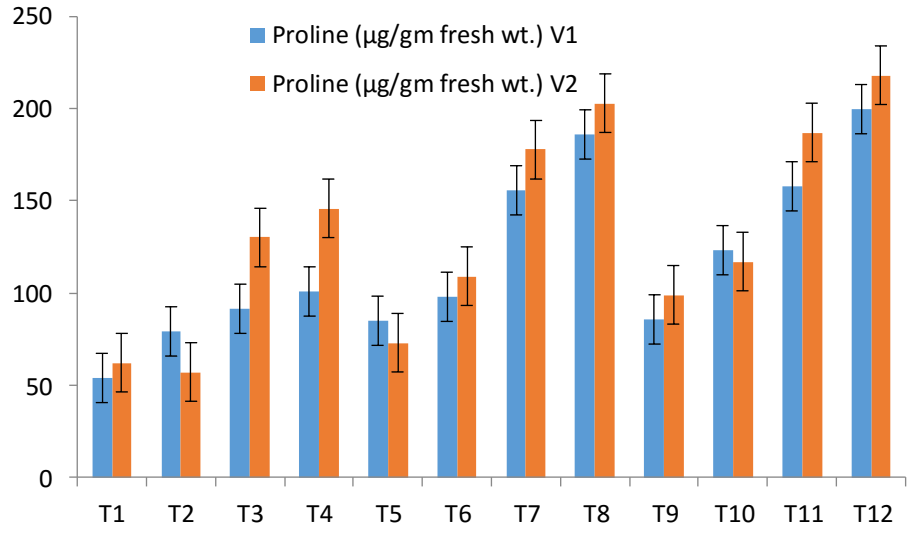

Figure 18: Impact of GB application on proline content of sunflower

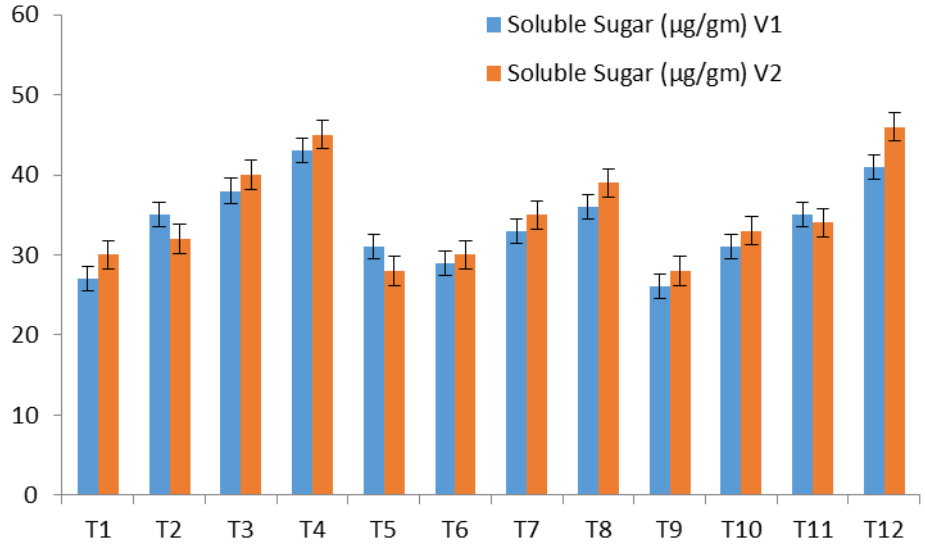

Figure 19: Impact of GB application on soluble sugar of sunflower

DISCUSSION

Drastic decrease in fresh weight of both hybrid plants was recorded under water deficit environment. As water stress considerably reduced the fresh weight of wheat crop (Rane *et al*., 2001), *Abelmoschus esculentum* (Rane *et al*., 2001) and pearl millet (Kusaka *et al*., 2005). The upshot for suppression cell growth and cell expansion due to the lower turgor pressure may results in such drastic decrease. Exogenous application of GB mitigates the stress effect and increased the fresh weight of sunflower hybrids at both the growth stages. Both hybrids have shown better results under irrigated mode of GB application as compared to foliar application.

The effect of irrigation levels was significant for dry weight of both the hybrid varieties under drought condition as water scarcity drastically decreased the dry weight of plants. This decrease in dry weight might be due to reduced photosynthetic activity, leaf senescence and plant growth (Rane *et al*., 2001; Kusaka *et al*., 2005).

Endogenously synthesized glycinebetaine improves the growth of above ground biomass following foliar application of GB. GB stabilize the turgor pressure and enzymes involved in amino acid metabolism, even at leaf concentrations upto 500 milliMole (Sankar *et al*., 2007). Laurie and Stewart (1990) reported that exogenous application of GB improved the growth in sunflower under drought and our results are also in accordance with above mentioned study. Several other reports clearly demonstrated the pronounced impact of exogenous application of GB on plant vegetative and reproductive stages in several crops e.g. maize (Agboma *et al*., 1997a) and soybean (*Glycine max*) (Hussain *et al*., 2008).

Better root system is fundamental adaptive mechanism that improves the ability of a plant to capture water towards drought stress. According to Agboma *et al*. (1997b) the root surface area in *Populus* species decreased in drought stress in the similar way the stem length in *Albizzia* decreased under water shortage (Agboma *et al*., 1997c). Lower turgor pressure reduce the plant height, this reduction in plant height might be associated with decreased cell enlargement and cell growth under stress (Sundaravalli *et al*., 2005).

The economic yield of crops can be estimated by the rate of photosynthesis. Drought stress decreases the photosynthetic activity. Similar results were obtained in present study. However, inhibitory effect of drought stress was ameliorated by foliar application of compatible solute (GB). Exogenous application of compatible solutes increased the net photosynthetic rate and also has been reported in maize (Yang and Lu, 2005) and tomato (Munns and Tester, 2008). Under drought or salt stress, GB not only control the stomatal conductance but also maintain Rubisco activity and chloroplast ultrastructure (Lopez *et al*., 2002).

There was a considerable decline observed for chlorophyll content of both hybrid under drought stress. Chl *a*, Chl *b* and Chl *a* + *b* contents decrease under progressive drought stress in maize (Nawaz and Ashraf, 2007). Exogenous application of GB improved photosynthetic pigments and thus enhanced photosynthetic capacity in various crops and vegetables e.g., tomato (Raza *et al*., 2006) and wheat (Anjum *et al*., 2011).

All water relations were disturbed due to water shortage including, turgor potential leaf water potential, osmotic potential and relative water contents at both growth stages of sunflower (Anjum *et al*., 2011). RWC decline depicts loss of turgor that results in limited water availability resulting in loss of turgidity which stops or decrease the cell expansion process in crop plants. In present study decrease in RWC was observed under drought conditions. Exogenous application of GB reversed the effect of water stress on RWC. This is in accordance with previous finding (Iqbal, 2004; Farooq *et al*., 2008) who reported an increase in RWC in kidney beans by foliar application of GB under abiotic stress. Active lowering of osmotic potential is generally considered as an adaptation under drought to maintain turgor (Iqbal, 2004). A beneficial drought resistance character is osmotic adjustment which is adopted by green plants (Meek *et al*., 2003) at lower leaf water potentials (Iqbal, 2004). To cope up with the stress effect, foliar application of GB is used as an important tool and has strong potential to reverse the stress effects. GB increased the turgor potential of the plant cells by osmotic adjustment (Iqbal *et al*., 2008).

Reduction in protein synthesis and proline accumulation in many crop plants have been widely studied (Iqbal *et al*., 2008) and are of the several biochemical indices of water deficit injury. Reduction in protein contents was observed in our study during exposure of drought stress. In leguminous plants, soluble protein content in both leaves and nodules decreased as drought progressed with more drastic decline in nodule tissues. Drought stress produced drastic effect on the soluble protein contents of leguminous plants in both leaves and nodules but the effect was more severe in nodule tissues. Amino acids accumulation in cell sap is one of the adaptations under water scarcity. Amino acid contents showed an uplift in sorghum plants when exposed to moisture stress conditions (Yaday *et al*., 2005). Similar results were observed in present study when sunflower plants were exposed to water stress. Osmotic adjustment alleviates some of the hazardous water stress effects. Lv *et al*. (2007) reported the accumulation of GB for drought stress tolerance in plants and the accumulation was more effective in transgenic plants containing enhanced activity of GB accumulation as compared to wild plants.

Accumulation of proline is chief indicator of drought stress tolerance in higher plants (Iqbal, 2004). The proline contents of leaf was increased in both sunflower hybrids at both stages. Accumulation of proline in various parts of plants is reported earlier particularly in wheat crop. Elevated level of proline was also observed in vegetable crop *Abelmoschus esculentus* (L.) under drought (Sankar *et al*., 2007). Glycine betaine application under drought condition ameliorated the stress effect at both the stages by accumulating proline in leaves. Exogenous application of GB increased the soluble sugars contents in sunflower at both growth stages (Lin *et al*., 2002; Lv *et al*., 2007) similar results were observed in present study.

CONCLUSION

Exogenous GB application increases chlorophyll content and photosynthetic rate of sunflower under water stress. According to this study, foliar application proved to be more effective among the two modes of application that have been investigated in the present study. The concentration of 125 mM GB were more operative as compared to 75 mM GB . Growth of both sunflower hybrids was effected by drought stress and had shown improvement towards application of GB but the reproductive stage was more sensitive as compared to the vegetative stage. It is concluded that 125mM GB concentration could be applied exogenously at reproductive stage to ameliorate the adverse effects of water shortage.

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BIOACTIVITY AND PHYTOCHEMICAL EVALUATION OF SUNFLOWER (HELIANTHUS ANNUUS L.) LEAF EXTRACT

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is a major oil yielding crop globally. Besides plant species has been traditionally used against fever, cuts, wounds, pulmonary infections and coughs. The experiment was conducted to validate its medicinal valve. Sunflower seeds were sown in pots and plants growth was maintained under greenhouse conditions. At flowering stage leaves were collected, shade dried and ground to fine powder. Sunflower leaf extract was prepared by cold maceration technique. Methanol extract was tested for antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl method (DPPH assay). Cytotoxicity was carried out by using Brine Shrimp Lethality assay and phytotoxicity by Radish Seed Germination assay. Extract exhibited significant antioxidant activity with IC50 value 0.89 mg/ml. A mild cytotoxic while a strong phytotoxic effect was observed with LD50 value 15 mg/ml and 0.4 mg/ml respectively. Phytochemical analysis revealed alkaloids, flavonoids, total phenolics and sterols in extract. In conclusion Sunflower extracts is a good candidate for drug development and isolation studies should be carried out to separate the bioactive components.

Key Words : Sunflower, cytotoxicity, allelopathy, antioxidant, pulmonary infections

THE ESTIMATING DROUGHT STRESS TOLERANCES OF SUNFLOWER INBRED LINES UNDER CONTROLLED ENVIRONMENTAL CONDITIONS

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ABSTRACT

Drought is the most severe factor reducing yield in sunflower. Sunflower plants responses to drought with some processes and changes at morphological, anatomical and molecular levels mostly with decreasing photosynthesis. In this study, 9 inbred lines of sunflower were investigated to get information about drought responses of them. With this purpose forty-day old 9 inbred lines growth at controlled well watered conditions were exposed to drought for 10 days, recovery of 5 days followed. Fast chlorophyll a fluorescence kinetics was determined and analysed using JIP test. Pigment content (chlorophyll *a*+*b*), relative water content (RWC) and membrane damage (ELC) were measured for both drought and recovery treatments. The adverse effects of drought stress were observed on photosynthetic efficiency that obtained by photosynthetic performance index (PItotal), RWC and ELC of leaves. However, following rewatering, recovery was observed for all inbred lines at different level. 9 sunflower inbred lines were classified into three groups; tolerant, less tolerant and sensitive, according to the injury index.

Key Words : sunflower, drought tolerance, inbred lines, controlled environments

EFFECTS OF NAPHTHALENEACETIC ACID AND N6-BENZYLADENINE ON ANDROGENESIS IN *HELIANTHUS ANNUUS* L.

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ABSTRACT

The aim of this study was to investigate the effects of NAA and BA on androgenesis in sunflower anthers. Hybrid sunflower breeding line taken from Trakya Agricultural Research Institute was used as a material. 70 days old capitula were sterilized and then anthers obtained from different sized capitula and flowers were transferred to the MS medium including four different concentrations (0, 0.5, 1 and 2 mg/l) of NAA and/or BA. Anther cultures were incubated at photoperiod or continuous dark conditions. Observations showed that uninucleate microspores were available in flowers 3-4 mm in length. While maximum callus induction for photoperiod condition was 87% when MS medium including 2 mg/l NAA and 1 mg/l BA was used, it was 90% for continuous dark condition at the same medium. Both plant growth regulators had no effect on androgenesis when used alone. But the androgenetic stimulation was gradually rose when they used together with increasing concentrations. When only light effect on androgenesis taken into account, callusing was 26% and 12% for photoperiod and continuous dark conditions respectively. There was no regeneration when anthers were transferred to regeneration medium. Investigation on anther-derived callus showed that there were both haploid and diploid cells too in it. According to these results, it was proposed that callus were formed from microspores. Finally, it was determined that NAA, BA and light had significant effects on stimulating androgenesis in sunflower.

Keywords: *in vitro,* Sunflower, Anther Culture, Haploid Production

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important source of vegetable oil in the world and in Turkey. In Turkey, sunflower oil is often preferred as vegetable oil consumption. Therefore, the importance of sunflower is increasing in recent years. Hybrid varieties are generally used in sunflower agriculture. However, due to the use of the same gene source, it is approaching the upper limit of capacity of genetic productivity in varieties obtained using traditional breeding methods. It is only possible through the use of biotechnological methods to overcome this problem. Therefore, the studies in this context in recent years are getting important every day and new achievements are obtained every year.

In this study, anther culture which is one of the biotechnological methods for obtaining haploid plant has been studied. As known, while breeding process with traditional methods lasts 7-8 generation, it can be reduced to a single generation using double-haploid technology. In this context, with the aim of promoting androgenesis in the anthers of sunflower (Helianthus *annuus* L.), the effects of different hormone combinations have been investigated. And, cytological observations were performed to determine the ploidy level of obtained calli.

Choi (1991), argued that optimal levels of hormones are needed according to the physiological status of anther tissue for androgenesis to reach success. Several specific plant growth regulators are used in anther culture. Generally, auxins such as 2,4-D, IAA, IBA, NAA, or alternatively, cytokinins such as kinetin, zeatin, and riboside were used in initiation and regeneration medium (Luckett and Darvey 1992). Vijaya Priya et al. (2003) has argued that for callus induction in wild sunflower species the presence of 2,4-D with auxin and cytokinin at low concentrations is sufficient but additional auxin and cytokinin is not necessary to increase the amount of callus. Similarly, despite the best callusing rates are obtained with the use of 0,5 mg/L NAA and BA in the culture of anthers derived from interspecific hybrids of the sunflower, when the concentration was increased to 1 mg/L, there was no significant changes in the rate of callusing (Nurhidayah 1996). On the other hand, Gurel and Kazan (1998) reported that increasing BAP concentration for all NAA concentrations was constantly increased the rate of callusing from different genotype of sunflower explants taken from different somatic tissues. Based on these information, the effect of NAA and/or BA (0 - 0,5 - 1 and 2 mg/L) on sunflower anthers for callus induction were studied. In Turkey, haploid culture studies on sunflower are less common and this study has been made in terms of creating a basis for these studies.

MATERIALS AND METHODS

In this study, hybrid sunflower breeding lines which has resistance gene to the orobanche and downy mildew was used. Seeds were obtained from Trakya Agricultural Research Institute. Sunflower capitula (figure 1) were collected from the plants of 70 days old. Flower buds (figure 2) 3, 4, 5 and 6 mm in length were isolated from capitulum of 3, 4, 5 and 6 cm diameter. The anthers removed from these flowers were analyzed by the asetocarmine squash method under Olympus photomicroscope. For sterilization Anthers were shaken in 15% commercial bleach for 20 minutes and for 2 minutes in 70% alcohol solution then rinsed with sterile distilled water three times. The sterile anthers were transferred to culture medium at photoperiod (16/8 light/dark) or continuously dark conditions. MS basic medium was combined with four different concentrations of NAA and/or BA (0-0,5-1-2 mg/L). 0,1% and 0,5% PVP added to the medium to prevent browning observed in Anther. The calli were transferred to MS medium which includes 0,5 to 1 or 2 mg/L BA and/or 0,1 mg/L NAA for regeneration experiments. Some calli are reserved for cytological examination in order to determine ploidy level of the cell. Fresh calli were analyzed by the asetocarmine squash method under Olympus photomicroscope. The differences among the averages of all experimental groups were tested by one-way ANOVA. In this test, the differences were compared with Tukey test at 0,01 significance level.

RESULTS AND DISCUSSION

Microscopic examination on the anthers obtained from 3, 4, 5 and 6 mm length of flowers was made with the aim of determining the appropriate microspores for successful anther culture (figure 3). It was observed that uninuclear microspores were seen in flowers of 3 and 4 mm length. Meriç (2002) reported that 3, 4 and 4.5 mm in length sunflower flowers are in the consistent with our preliminary investigations. Therefore, in this study with the aim to obtain microspores in the uninucleate stage, anthers were provided from flower of 3 and 4 mm in length. Additionally, according to our study, it was observed that the flowers of 3-4 mm in length located in the uninucleate stage, but 5.5 cm and up in length are in the binucleate stage. The literature is capitulum of 3 to 4 cm in diameter.

Figure 1. **Sunflower capitula**

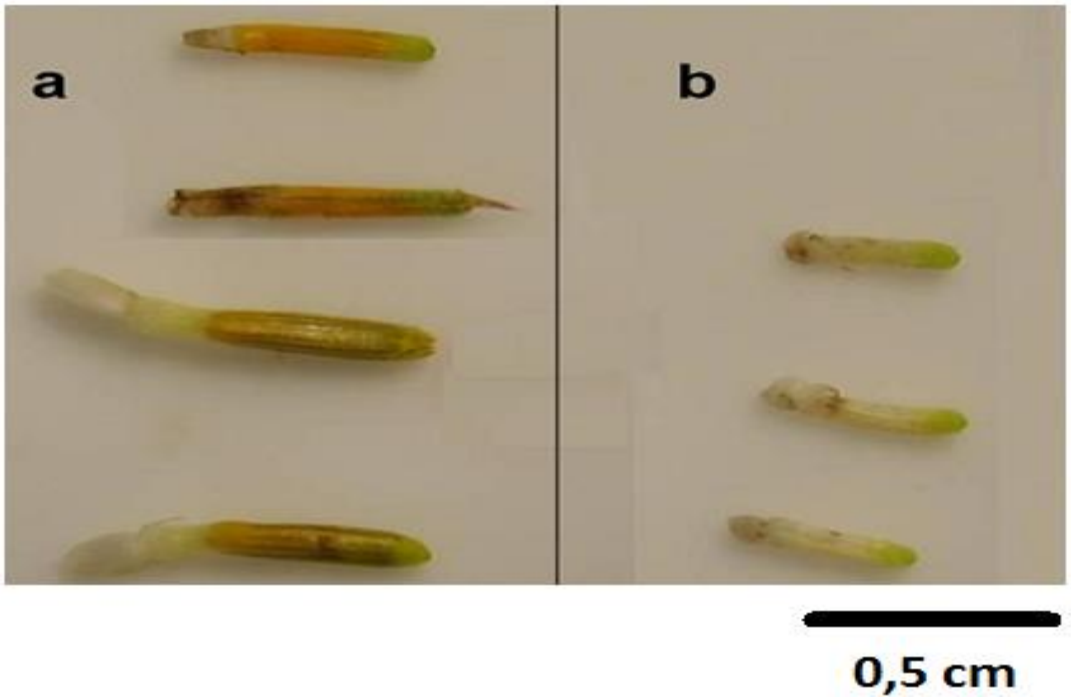


Figure 2. Sunflower tubular flowers. a) 5-6 mm in length, b) 3-4 mm in length

One of the most evident result of our study, any micspor response has not occurred in hormone-free basic MS medium (Table 1). All anthers in this medium browned and lost their vitality after 2 weeks even they showed some swelling at first. Vijaya Priya et al. (2003) studied on six different sunflower genotypes and reported that callus formation was not observed in hormone-free MS basic medium as similar to our findings. They explained this case by idea of the endogenous hormone levels in the anthers were not enough the promote callusing. We agree with this idea because of androgenic response does not occur in hormone - free MS medium in our study.

Table 1. Effect of NAA and BA on Callusing.

Light Regime	NAA mg/L	BA mg/L	Callusing %*
Photoperiod	0	0	0 a
"	0	0,5	0 a
"	0	1	0 a
"	0	2	0 a
"	0,5	0	0 a
"	0,5	0,5	7 a
"	0,5	1	27 abcd
"	0,5	2	47 cde
"	1	0	0 a
"	1	0,5	0 a
"	1	1	47 cde
"	1	2	50 def
"	2	0	0 a
"	2	0,5	73 efg
"	2	1	87 g
"	2	2	77 fg
Continuously Dark	0	0	0 a
"	0	0,5	0 a
"	0	1	0 a
"	0	2	0 a
"	0,5	0	0 a
"	0,5	0,5	0 a
"	0,5	1	20 abc
"	0,5	2	20 abc
"	1	0	0 a
"	1	0,5	0 a
"	1	1	0 a
"	1	2	7 a
"	2	0	0 a
"	2	0,5	40 bcd
"	2	1	90g
"	2	2	13 ab

*Values within each column followed by the same letters are not significantly different by the Tukey test at 0.01% probability level.

Another important result is that when NAA or BA used alone, there was no callus formation from anther (Table 1). On the other hand, when NAA and BA used together callus formation was observed in anther after two weeks (figure 4). Moreover, when NAA and BA concentration increased, the rate of callus formation is increased significantly. The best callus formation ratio (90%) was obtained on MS basic medium consisting of 2 mg/L NAA and 1 mg/L BA after 6 weeks (figure 5). Vijaya Priya et al. (2003), in their study, when 0.1 mg/L

NAA and 0.2 mg/L BA is added on the MS medium of, callus formation was observed depending on genotype ranged from 77% to 97%. When the rate of growth regulators are increased as 2.0 mg/L NAA and 1.0 mg/L BA, callus formation rates ranging still from 90% to 74% depending on the genotype was observed. They reported that the increasing concentrations of auxin and cytokinin (NAA and BAP) do not have a significant effect on the callusing rate. Similarly, Nurhidayah (1996) reported in his study that, the increasing concentrations of NAA and BA do not cause a significant increase in callus formation. These findings contradict with our findings. On the other hand, Gurel and Kazan (1998) reported that increasing BAP concentration for all NAA concentrations was constantly increased the rate of callusing from different genotype of sunflower explants taken from different somatic tissues. Although Vijaya Priya et al. (2003) has been reported that optimum doses of growth regulators are enough to callus induction and the increase of the dose does not influence the callusing rate, we claim that increasing the concentration of NAA and BA, increases callus formation rate based on the sunflower genotypes we used. As the different genotypes are used in each different study, this is believed to be the cause of these conflicts. Different genotypes and incubation conditions (constant light-dark-photoperiod) have various effects on callus formation. Considering the interaction between these factors, if the target is androgenetic stimulations in anthers of sunflower, our proposal is that concentration and combination of growth regulators must be optimized separately for each genotype and incubation condition.

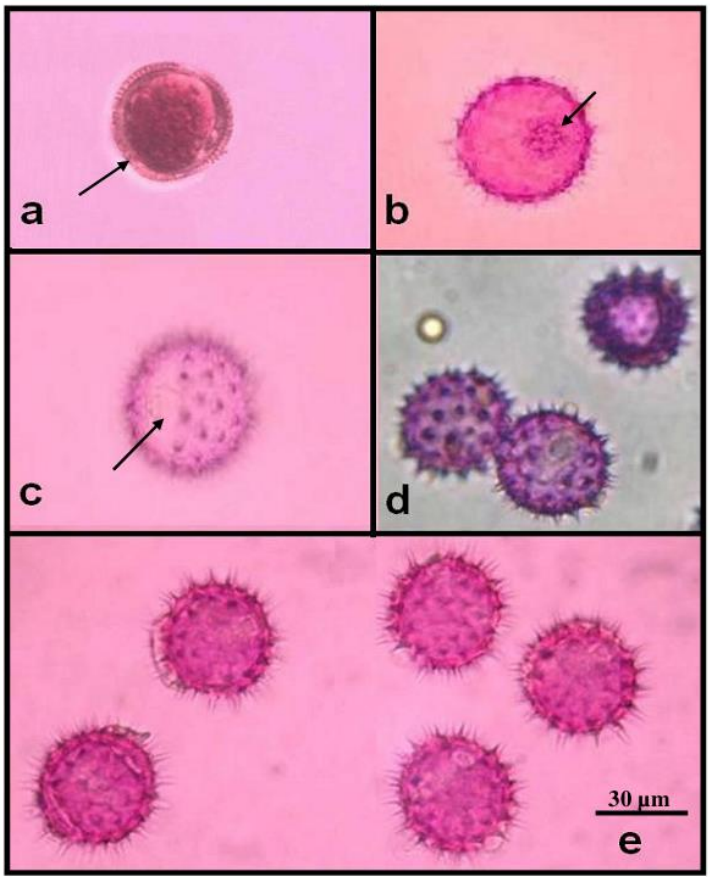


Figure 3. Sunflower microspores and pollens. a) early-uninucleate microspore stage b-c) late-uninucleate microspore stage d-e) binucleate pollens

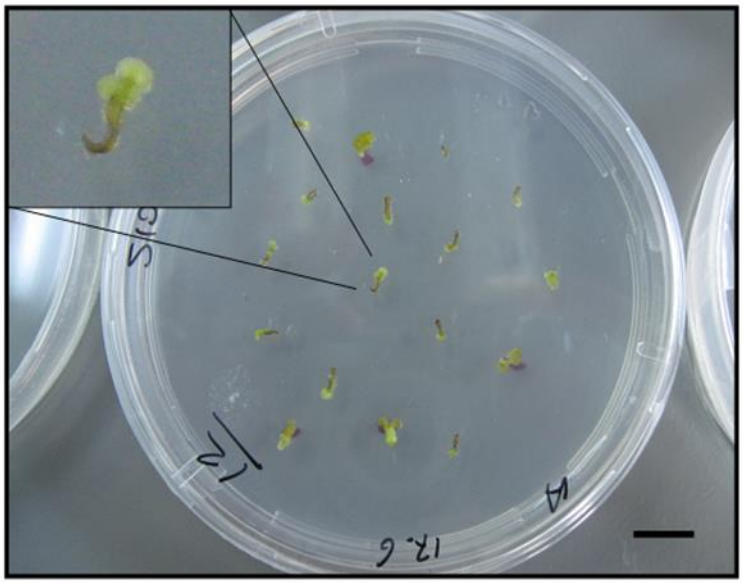


Figure 4. Second week of anther culture. MS medium includes 2 mg/L NAA and 1 mg/L BA. Bar=1 cm.

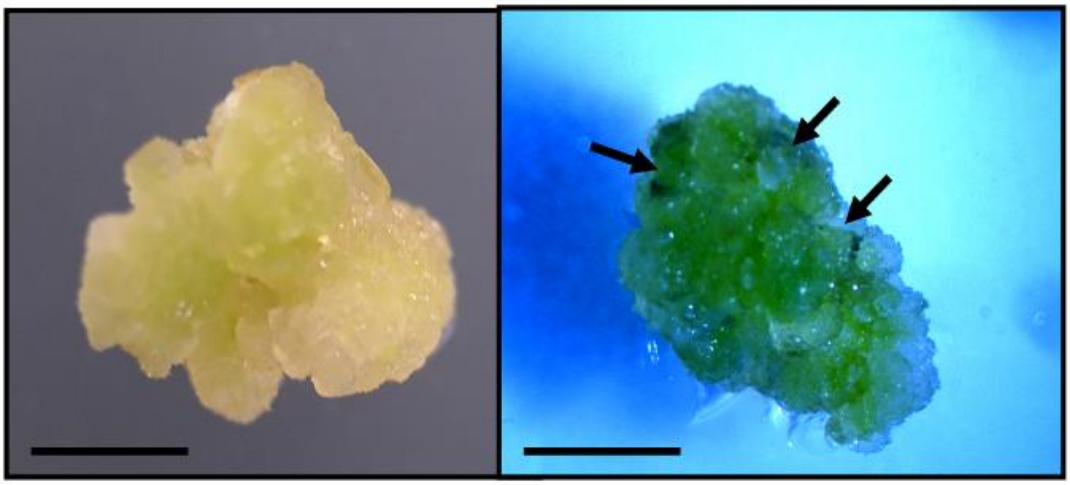
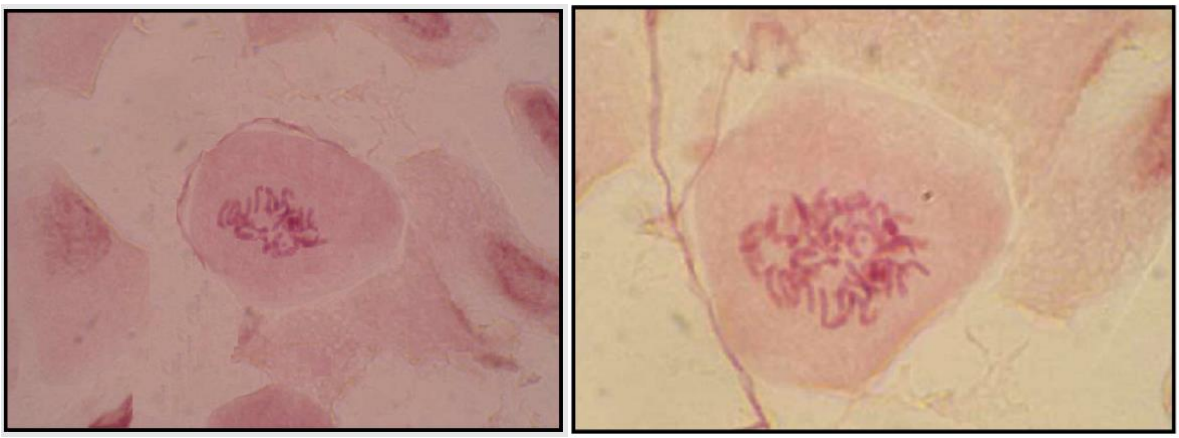


Figure 5. Sixth weeks old calli on MS medium includes 2 mg/L NAA and 1 mg/L BA. Yellowish callus occurred in dark condition on left, and green callus occurred in photoperiod condition on right. Bar=1 cm.

Previous studies have reported that by adding antioxidants such as PVP to the medium prevent browning (Roy and Sarkar 1991; Sudripta et al. 1999). In this study four different concentrations of PVP were tested with the reason of overcome the browning problem occurred in anther, but there was no statistically significant effect in terms of PVP. There was no shoot regeneration from callus in any experiment group. It is necessary to keep in mind that the low productivity is common in shoot regeneration from anther culture (Thengane et al. 1994). When the literature is studied it is outstanding that this case is common. Bohorova et al. (1985) has achieved anther callus around 70-100%, but failed to obtain shoot regeneration. Gürel et al. (1991a) reported low frequency direct embryo formation and shoot regeneration in the sunflower anther culture, but a whole plant could not be obtaioned. Gürel et al. (1991b) and Coumans and Zhong (1995) tested the isolated microspore culture and even they obtained continuous cell division and microcallus formation, they could not achieve shoot regeneration. A total of 200 callus have been examined, but chromosomes could be counted in small number of cells. However, both haploid and diploid cells were also observed in the callus (figure 6). The observation of haploid cells indicates that calli are microspore-derived. It is believed that diploid cells are spontaneous double-haploids as well.

Figure 6. Microscopy of sunflower callus. Hapliod (on left) and diploid (on Right) cells.



As a result, in this study it was found that the NAA should be used together with the BA to stimulate androgenesis from sunflower anther derived from flowers 3-4 mm in length. It is essential to product double-haploid plants originated from anther to contribute the sunflower breeding work in Turkey. Therefore, we hope the results of this study will be a guide for future studies.

This research which is the part of Sergun DAYAN's doctoral thesis was supported by Trakya University Academic Research Projects Unit (Project number: TÜBAP 2009-92).

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CYTOKININS: THE KEY TO DIFFERENCES IN PATTERNS OF CANOPY SENESCENCE IN STAY-GREEN AND FAST DRY-DOWN SUNFLOWER HYBRIDS

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ABSTRACT

This study documents the dynamics of cytokinin levels in leaves and their association with root functionality and leaf senescence in irrigated crops of two sunflower hybrids with different patterns of leaf senescence (stay-green[SG] and fast dry-down[FDD]) grown in two years. During the grain filling-phase, green leaf area index (GLAI) and live root length density (LRLD) were followed, together with total chlorophyll content (CT), fluorescence (Fv/Fm), net photosynthesis (Pn) and trans-zeatin (ZT) levels in leaves of positions H17, H20, H22 and H24. In all positions, hybrids and years the beginning of leaf senescence was firstly associated with decreases in CT, followed by falls in Fv/Fm and Pn. Root senescence differed ($p<0.05$) between hybrids, where FDD always started first, and changes in LRLD preceding those of GLAI. ZT levels in leaves decreased ($p<0.05$) between active-phase and those in senescence-phase. At all positions, the beginning of decrease was later ($p<0.05$) and initial ZT levels were higher ($p<0.05$) in SG: 2.34(H17), 3.03(H20), 4.14(H22) and 7.96(H24) times higher than FDD leaves positions. The decrease per degree-day was 1.11%(H17), 0.63%(H20), 0.39%(H22), 0.58%(H24) of initial values in FDD and 0.79%(H17), 0.72%(H20), 0.27%(H22), 0.64%(H24) in SG. Differences in leaf senescence between SG and FDD were mainly associated with initial ZT levels in leaves. These results are the first to describe variations of leaf cytokinin levels during leaf senescence in sunflower (and other cultivated species), suggest that beginning of leaf senescence is inversely related to leaf ZT levels, and demonstrate that root senescence precedes that of leaves.

Key Words : Canopy senescence, Chlorophyll content, Cytokinins, Leaf senescence, Root senescence, Sunflower, Trans-zeatin levels

PHYSIOLOGICAL BASIS AND ANTIOXIDANT ACTIVITY IN COLD STRESS RECOVER IN SUNFLOWER (HELIANTHUS ANNUS L.)

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ABSTRACT

Tolerance to low temperatures is an important trait, considering that the sunflower production area is expanding to marginal regions with suboptimal growing conditions, and there is an increasing requirement of early sowing to maximize the growing season over Mediterranean areas in countries such as the United States of America, India and Argentina. The present study of the response of sunflower to low temperatures focused on the primary responses on young plants after 96 h under cold treatment at 5°C with the aim of detecting regulatory mechanisms induced at this early stage. Studying the antioxidant activity and physiological bases involved in recovery from cold stress in sunflower seedlings may allow these characteristics to be used in breeding programs aimed at selecting varieties of sunflower adapted to stress from sub-optimal temperatures. The purpose of this research was to establish the recovery from cold stress in terms of the activity of the antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), and its relationship with the physiological response of two sunflower hybrids to the contrasting response to cold stress. Prior studies in the Plant Physiology Laboratory identified two sunflower hybrids with contrasting response to cold at the germination stage: sensitive the hybrid *Pampero*(PM) and tolerant the hybrid *Sierra Alto Oleico* (SA). Ten day old seedlings of commercial hybrids PM and SA were placed in cold storage for 96 hours at 5°C, and cold stress recovery was assessed in terms of the following variables: Level of Damage to Cell Membranes through the content of malondialdehyde (MDA), Antioxidant Enzyme Activity: Superoxide Dismutase (SOD) and Catalase (CAT), and Chlorophyll Content at 0, 24, 48 and 72 hours after exposure to cold. In addition, Total Plant Dry Mass and Leaf area were determined per plant. The response to cold stress was greater in the SA than the PM hybrid, suggesting that the former possesses repair mechanisms at the cell level which are activated more quickly in response to low temperature. This coordination and activity levels of the enzymes SOD and CAT found in this study in SA are in accordance with the lower level of cell damage (observed in lower MDA levels), as compared to PM. Higher antioxidant activity and lower MDA levels allow sunflower plants to maintain their photosynthesizing apparatus active, maintaining the functionality of chlorophyll for dry matter production, and leaf area during the early stages of growth, after exposure to cold stress. All the variables described here may be used as criteria for screening cold-stress tolerant sunflower genotypes.

Key Words : sunflowers genotypes, cold stress recover, abiotic stress, physiological traits, oxidative stress, antioxidant defence.

EXPRESSION OF DEFENSE RELATED GENES IN LEAVES OF TWO SUNFLOWER LINES AFTER INFECTION WITH SPORES OF PLASMOPARA HALSTEDII

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ABSTRACT

Two sunflower lines, susceptible and resistant to downy mildew race 730 were used in this study. Susceptible line was Ha-26 and resistant line was its backcross BC/8 analogue, containing Pl6 gene for downy mildew resistance introduced from initial cross with Ha336. Inoculation with the suspension of *P. halstedii* zoospores, was done on the plants in the phase of first pair of leaves. Twelve days after infection susceptible line developed typical disease symptoms, i.e. leaf chlorosys with or without sporulation, which was not the case with resistant line. In the time period of 2 to 96 hours after treatment leaves were harvested and immediately frozen in liquid nitrogen. Total RNA was isolated by RNAeasy kit (Quiagen). cDNA synthetised by RevertAid First Stand cDNA Synthesis Kit (Fermentas), was used as template in PCR to examine the expression pattern of several defense related genes. The expression of genes for enzymes involved in H2O2 production (Caox; Oxox) was constitutive but significantly higher in resistant line, already 2 h after infection. Similar results were obtained for SODc gene. Higher accumulation of SODp and chitinase transcripts was observed up to 4h after infection in resistant line. PR5 transcript was upregulated in early phases after infection only in resistant line. Our results indicate that the early response to secondary downy mildew infection resembles to hypersensitive-like reaction and is partly responsible for the resistance conferred by Pl6 gene.

Key Words : downy mildew, Pl6, gene expression

A SOURCE-SINK BASED DYNAMIC MODEL FOR SIMULATING OIL AND PROTEIN ACCUMULATION IN SUNFLOWER ACHENES

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ABSTRACT

The potential interest of a dynamic crop model is to provide reliable predictions of oil concentration (OC) soon before harvest as well as helping to understand at which time oil dynamics was affected by environmental stresses or management. For that purpose, we proposed a "source-sink" based dynamic model describing on a daily step nitrogen and carbon assimilations and remobilizations during sunflower grain filling. Priority rules were established for C and N depletion from "source" organs, as well as for their allocation into "sink" organs. Photosynthesis was simulated using the radiation use efficiency approach and nitrogen uptake according to Pan *et al.* (2006) formalisms. Water and N stresses were computed by SUNFLO crop model from climatic and soil data and genotype characteristics. The "source" and "sink" variables were initialized at flowering and main outputs were oil and protein concentrations and weights per m². The model was calibrated on 24 crop situations in 2012 and evaluated independently on 50 other situations (3 years) with contrasted genotypes and environments. Global trends were well reproduced for all "source" and "sink" components but most variables tended to be overestimated. The main indicators of model quality for predicting OC were: RMSE = 6.1 (%), efficiency = 0.97, R^2 = 0.94 and Bias = -0.06 (%). A sensitivity analysis suggested us to reduce the number of parameters, better describe photosynthesis and N uptake processes, and improve the parameterization of genotype and nitrogen effects in order to decrease the prediction error and provide a relevant tool for OC prediction.

Key Words : source-sink model, seed oil content, seed protein content, C-N remobilization, C-N assimilation

MORPHOANATOMY OF INCOMPLETELY DEVELOPED FRUITS IN THE SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

The occurrence of fruits with poorly developed embryo or absent, usually defined as empty, seedless or incompletely developed fruits (IDF), significantly reduces the sunflower yield. The morphology and anatomy of ovaries and fruits sampled 10 and 20 days after anthesis (DAA) and at physiological maturity of Dekasol 3940 were analyzed. Samples were taken on different positions around the capitulum close to the midpoint of the radius. The percentage and size of IDF were similar between sampling dates and capitulum positions. The dimensions of the IDF with black pericarp (IDFBP) and filled fruits (FF) were similar, indicating that pericarp development had finished 10DAA. The width (20%) and thickness (46-57%) of IDF with white pericarp (IDFWP) were lower than IDFBP. 10DAA, 17% of IDFWP presented embryos while the remaining enlargement of the embryo sac and proliferation of integumentary tapetum. The IDFBP presented a developed endosperm with (78%) or without (22%) embryo. At 20DAA, the embryo width and length in IDF did not exceed that of the FF at 10 DAA. The results indicated that IDF were generated by embryo abortion within 10DAA and not from any kind of fertilization failure. The percentage of IDF correlated with FF weight (r = -0.84 **) and capitulum diameter (r = -0.76 **).

Key Words : Sunflower, Empty Fruits, Ovary and fruits anatomy.

LIGHT DEPENDANT BIOSYNTHESIS OF SESQUITERPENE LACTONES IN SUNFLOWER

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ABSTRACT

Asteraceae are rich in sesquiterpene lactones (STL), compounds known to elicit various bioactivities and to serve particularly as protectants against herbivory. In sunflower, STL primarily occur in capitate glandular trichomes (CGT) from where enzymes of their biosynthesis were characterized. In inner tissues, STL are ca. 10.000-fold less concentrated and have completely different function. They probably are involved in light dependent growth reactions. Here we report on the CGT development and STL biosynthesis on leaflets under different light qualities related to cryptochrome and phytochrome. CGT cell division took place between 48 and 72h after seedling germination. Afterwards, STL were produced and secreted into an external cuticular globe. Trichome formation was independent from light quality or intensity applied during germination. However, STL synthesis and expansion of the cuticular globe was triggered by near red (660nm) and blue light (465nm) irradiation during cultivation, whereas far red light (730nm) and darkness did not lead to significant STL accumulations. Short pulses of near red light versus continuous irradiation lead to similar amounts of STL. Moreover, far red light pulses extinguished effects of near red light pulses, thus indicating phytochrome regulation of STL in trichomes. Near red light also influenced the synthesis of the STL 8-epixanthatin in inner sunflower tissues. Compared with seedlings grown in darkness or far red light, near red light increased the STL amount in roots of seedlings 5-fold or more. Internal STL seem to be involved in tropisms. Moreover, they leak into the rhizosphere where they induce germination of broomrape.

Key Words: Trichomes, terpene synthesis, herbivory, phototropism, phytochrome, cryptochrome, sunflower broomrape

LEAF SENESCENCE IN SUNFLOWER WAS ADVANCED OR DELAYED DEPENDING ON CHANGES IN THE SOURCE-SINK RATIO DURING THE GRAIN FILLING PERIOD

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ABSTRACT

Lifespan of leaves is usually associated to grain yield in sunflower. During the grain filling period, demand of grains converges with a decreasing green coverage. The source-sink ratio (SSR) allows estimating a carbon budget between the organs vegetative and reproductive of the plant. The aim of this work was to study the effect of the source-sink ratio during the grain filling period on the leaf senescence in sunflower. Sunflower hybrid VDH 487 was sown in a field trial, and maintained in good conditions of water and nutrition. The SSR was increased in three moments (head removal), and decreased in one (shading), during the grain filling period. Senescence was evaluated from the decrease in the levels of chlorophyll, fluorescence, carbohydrates and dry matter, in leaves 15, 20 and 25 (from the bottom). The progress of all studied variables was advanced about 175°Cd in leaf 15 in comparison with leaves 20 and 25 (p<0.05). The increase in the SSR delayed leaf senescence measured trough all variables studied in this work up to 400°Cd, depending on the leaf number and the variable itself. In general terms, delay was greater when plants were submitted to longest periods of high SSR. Conversely, a decrease in SSR advanced senescence. Depending on the leaf and the studied variable, this advancement attained up to 256°Cd (for the decrease in carbohydrates content). In this work, we verified, by manipulating SSR, that leaf senescence is not fixe in time. As in most monocarpics, grain filling played an essential role in senescence.

Key words: Sunflower, Senescence, Source sink ratio, Grain filling period

INTRODUCTION

Lifespan of leaves is usually associated to grain yield in sunflower. Senescence is a genetically programmed process which produces cell disruption and finally its death after nutrient recycle to younger or storage tissues (Gan and Amasino, 1997; Buchanan-Wollaston et al., 2003). It is influenced for both genetics and environmental variables. As it generally occurs at the end of the lifespan it is associated to a decrease in photosynthetic activity, macromolecules degradation, loss of chlorophyll, decrease in nitrogen content, etc. (Smart, 1994; Buchanan-Wollaston, 1997). Some, or all, of these processes can be used as traces of senescence progress (Moschen et al., 2012). Loss of chlorophyll is probably the feature the most used to characterize leaf senescence, but carbohydrates and photosynthesis are also mentioned in literature (Crafts-Brandner et al., 1984; Sadras et al., 2000).

Reproductive growth in annuals is related to leaf senescence since senescent leaves increase after flowering (Thomas and Stoddart, 1980; Gan and Amasino, 1997; Yoshida, 2003). Thus, demand of grains converges with a decreasing green coverage during the grain filling period.

The source-sink ratio (SSR) allows estimating a carbon budget between the organs vegetative and reproductive of the plant. Imbalances of SSR can accelerate leaf senescence (Rajcan and Tollenaar, 1999). In oilseeds species, including sunflower, experiments preventing grain formation showed a delay in leaf senescence (Lindoo and Nooden, 1977; Ho et al., 1987). In maize, there are contradictory findings in literature. While in most works an increase in the SSR resulted in advanced leaf senescence (Rajcan and Tollenaar, 1999; Allison and Weinmann, 1970), some others showed an advance or a delay in leaf senescence (Thomas and Smart, 1993). In all of these cases, carbohydrates metabolism plays a principal role (Wingler et al., 2006).

During the grain filling period both the source and the sink adjust their relative magnitude in response to environmental and own of the plant factors. Thus, not only SSR could change as it progress through the period, but we can hypothesize that it effect on leaf senescence could also be modified. No reports were found about the effect of the changes in SSR in different moments of the grain filling period on leaf senescence.

The aim of this work was to study the effect of the source-sink ratio during the grain filling period on the leaf senescence in sunflower.

MATERIALS AND METHODS

The experiment was conducted at the INTA Balcarce Experimental Station, Argentina (37° 45´S, 58° 18´W) during the 2012/13 season. Hybrid VDH 487 (Advanta) was sown the 22 Oct. Emergence occurred 9 days after sowing. Plant density was 5.7 plants m-2. The experiment was conducted under good nutritional and water conditions. Weeds and insects were controlled. Phenological stages were recorded according Schneiter and Miller (1981). Flowering occurred the 9 Jan. and physiological maturity the 12 and 16 Feb. in control and shading treatments, respectively. Time was expressed on a thermal time basis by daily integration of air temperature with a threshold temperature of 6 ◦C (Kiniry et al., 1992). The following treatments were applied in a factorial combination of different levels of source-sink ratio (SSR) in a randomized block design with three replicates: (i) head removal in stages R6, R7 and R8, (ii) 50% reduction of solar radiation in R7 stage by shading, and (iii) untreated control. Each plot consisted of four 10m long rows spaced at 0.7m. Daily mean temperature and solar radiation and rainfall were measured in a weather station located 400 m from the experiment. Target leaves (15, 20 and 25 from the bottom) were selected based on the representativeness of the profile of the plant.

Leaf initiation in the apex was measured every 48 to 72h from emergence after apex dissection under stereoscopic microscope of three plants *per* plot. Results from the progress in time of the number of leaves were adjusted to a linear regression model, from which the initiation date at the apex of target leaves was estimated.

Dry matter of target leaves was measured every 15 days in three plants per plot. Samples were cut from the plant and oven-dried (with air circulating at 60 ◦C) to constant weight and weighed.

Soluble carbohydrates were measured every 15 days according to Dubois et al. (1956), from a 50mg sub sample of leaves dry matter after milled. Absorbance at 490nm from both the sample and the glucose standard was measured with an spectrophotometer (Bausch & Lomb, Spectronic 20, USA). 19th International Sunflower Conference, Edirne, Turkey, 2016 Quantum yield of PSII was measured every 10 days with a handheld fluorometer (FluorPen FP100 Z990, Photon Systems Instruments, Drasov, República Checa).

Chlorophyll was measured every 5 to 16 days after extraction with 2,3 N, N dimetilformamida from 6 discs of 0.5 cm diameter taken from each leaf. Absorbance at 664.5nm and 647nm was measured with a spectrophotometer (Bausch & Lomb, Spectronic 20, USA). Chlorophyll content was estimated according Inskeep and Bloom (1985).

The timing of the evolution of each of the variables in which its value fell to 80% of its maximum value was considered as an indicator (event) of the onset of senescence evaluated with that variable in each target leaf. In the case of chlorophyll are also considered the 20% ("yellowing") and 0% ("dead"). The occurrence of these related to senescence events, was ordered on a unique thermal time scale. The sequence of events was considered modified when the occurrence of at least two of them was reversed compared to the sequence set in the control (for evaluation of SSR effect) or compared to any of the remaining two leaves (for evaluation of the effect of leaf age).

Data of dry matter, carbohydrates, chlorophyll and quantum yield in each sample date were processed by analysis of variance procedures. Sequences of senescence related events were assessed by the method of analysis of variance using an unique model including treatments and leaves. Differences between the mean values were evaluated from the test of least significant difference (LSD, p <0.05). All analyzes were performed with the statistical analysis program Infostat Professional v.1.1 (Di Rienzo and Robledo, 2002).

RESULTS

Meteorological conditions during the experiment*:*

Daily mean temperature during the experiment was 1ºC higher than the historical average, being lower only in March. Contrarily, daily mean radiation did not differ significantly from the historical (18.3 vs. 18.5 MJ.m-2.d-1). In December and January, radiation in 2012-13 was lower than the historical, while in February it was higher (Table 1). Rainfall was higher than the historical, especially in December and January (134% and 40%, respectively, Table 1).

Table 1: Daily mean temperature and solar radiation and rainfall during the months of the experiment (2012/13), and for an historical series (H) of 41 years (1971-2011).

	Temperature (ºC)		Solar radiation ($MJ.m^{-2}.d^{-1}$)		Rainfall (mm)	
	2012/13	H	2012/13	H	2012/13	H
Nov-12	17.7	16.0	21.7	21.1	64	89
Dec-12	20.2	18.8	21.1	22.0	239	102
Jan-13	21.1	20.2	20.6	22.0	152	108
Feb-13	21.2	19.8	21.1	19.5	33	85
Mar-13	16.4	18.1	15.3	15.2	114	92
Apr-13	17.2	14.4	9.8	10.9	74	80

Characterization of the effect of treatments on the target leaves.

Dry matter:

In leaf 15, after the application of R6 and R7 treatments the loss of dry weight was delayed, while in S it was not affected (Fig. 1.A). In leaves 20 and 25, head removal produced an increase in dry matter (R6) and/or the delay in weight loss in advanced stages (R6, R7 y R8).

Shading accelerated weight loss (Fig. 1 B y C). The weight of the leaves towards the end of the cycle was higher as the demand was interrupted before (R6>R7>R8>C).

Chlorophyll:

After head removal in R6 and R7 a delay in chlorophyll degradation was observed in leaf 15 (75% between R6 and C at 1068°Cd, $p \leq 0.05$, Fig. 1.D). Maximal chlorophyll content in leaf 20 was near 0.04 mg/cm2. Head removal also delayed chlorophyll degradation in this leaf, while in S it was advanced (Fig. 1.E). The same was observed in leaf 25, although degradation in S was faster than in leaf 20 (Fig. 1.F).

Quantum yield of PSII (Qy):

Head removal in R6 and R7 delayed decrease in Qy in leaf 15 (Fig. 1.G). In leaf 20, head removal in R6 maintained maximal Qy 400°Cd more than C (Fig. 1.H). Treatment S advanced Qy fall, which attained 0% near 200°Cd before the control (Fig. 1.H). Treatments R6, R7 and R8 in leaf 25 delayed 100°Cd the decrease in Qy (Fig. 1.I). Conversely, S treatment advanced more than 200°Cd the falling of this variable, presenting at 1200°Cd a Qy 65% lesser than the control ($p \leq 0,05$, Fig. 1.I).

Soluble carbohydrates (HCS):

Head removal in R6 allowed maintaining maximal HCS concentration more than 100°Cd in leaf 15. When treatment was applied in R7, HCS of the C was already reduced to the half of the maximal value; anyway it delayed the falling more than 100°Cd comparing to C (Fig. 1.J).

In leaf 20, head removal allowed in the three treatments (R6, R7 y R8) maintained higher concentration of HCS for a longer period than C, and in the cases of R6 and R7 also delayed the start of the decrease. Conversely, in S advanced more than 300°Cd both the start and the end of the drop in HCS (Fig. 1.K). In leaf 25 the response was rather similar to that of leaf 20 (Fig. 1.L).

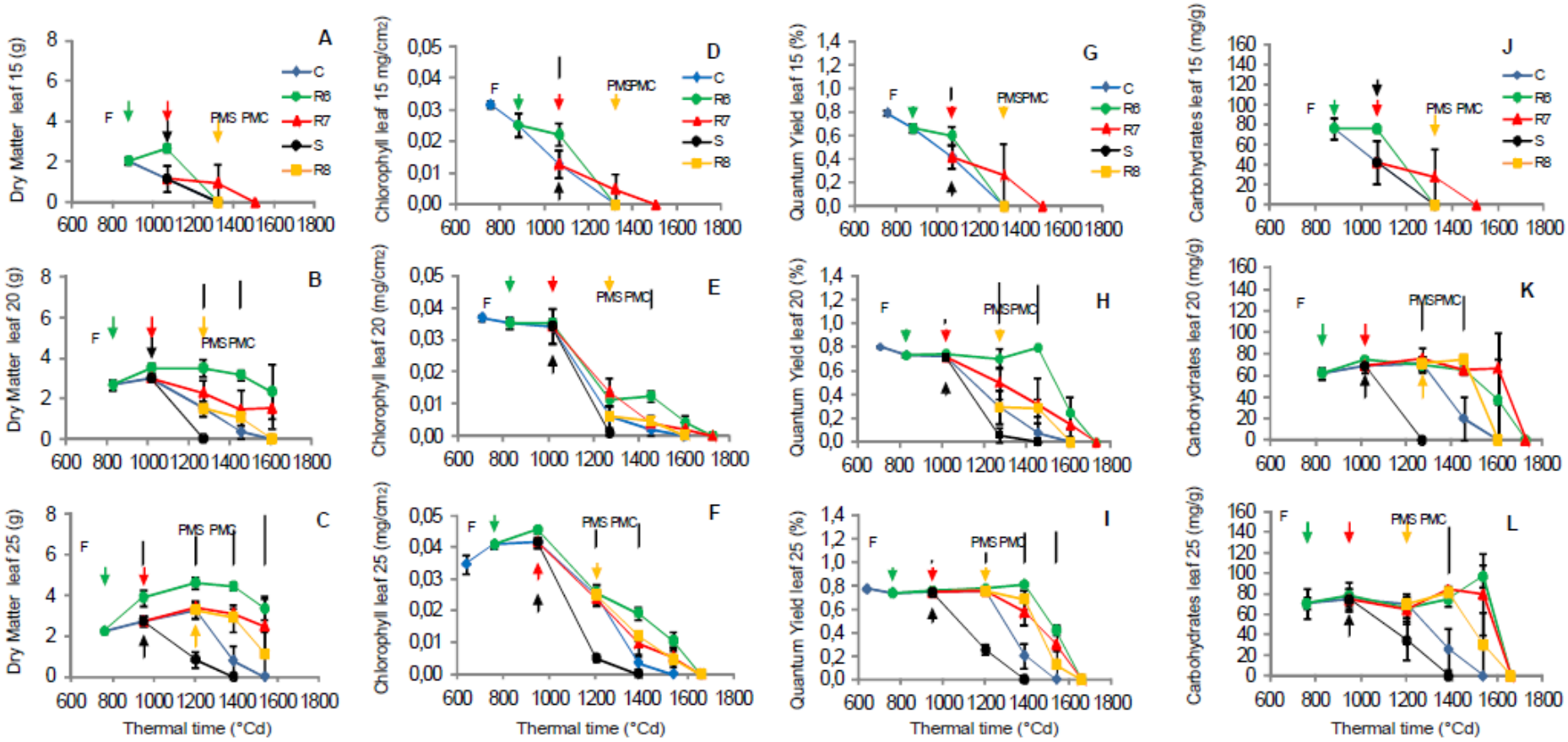

Fig. 1 Dry Matter (A-C), Chlorophyll content (D-F), Quantum yield of PSII (G-I) and Carbohydrates (J-L), in Leaf 15 (A, D, G, J), 20 (B, E, H, K) and 25 (C, F, I, L) as a function of the thermal time after their initiation in the apex. Treatments: control: C, head removal in the developmental stages: R6, R7 and R8, and shading reducing 50% of incident radiation: S, in R7 stage. Vertical lines on the symbols are the standard error of the mean value (n=9). Vertical lines above indicate the least significant difference for each sample date (α=0.05, LSD). Arrows indicate treatment application. F means flowering, and PMC and PMS, physiological maturity in C and S treatments.

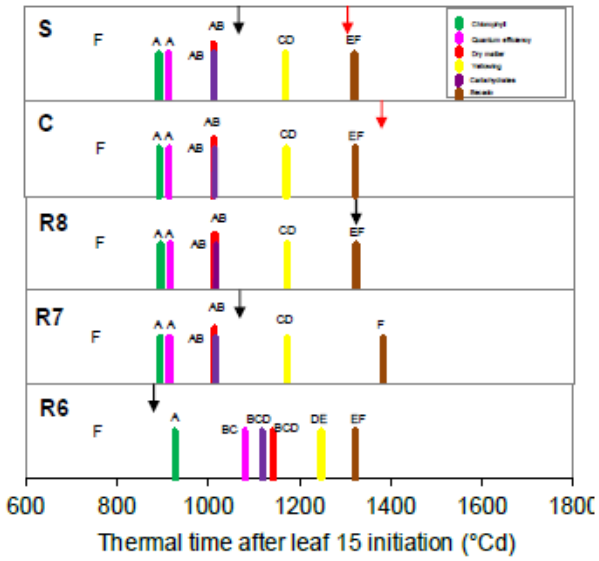

Fig. 2 Events related to leaf senescence sorted in a thermal time scale from the initiation of leaf 15 in the apex. Different letters indicate that 2 events from the same or a different treatment are significantly different (α=0.05). Arrows indicate treatment application (black) or physiological maturity (red). F indicates flowering date.

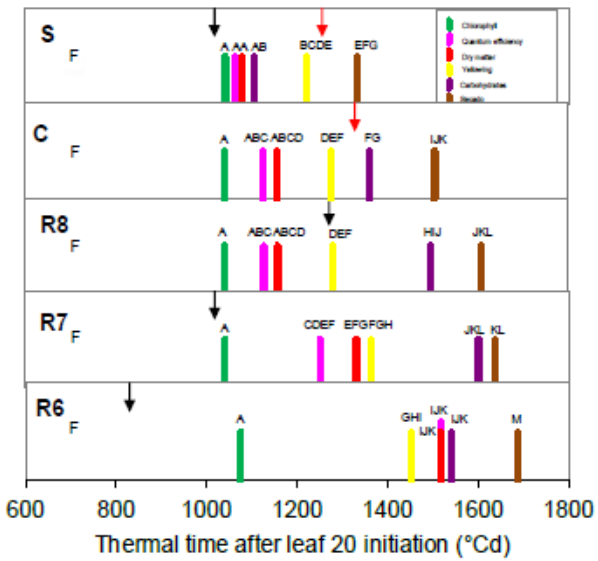

Fig. 3 Events related to leaf senescence sorted in a thermal time scale from the initiation of leaf 20 in the apex. Different letters indicate that 2 events from the same or a different treatment are significantly different (α=0.05). Arrows indicate treatment application (black) or physiological maturity (red). F indicates flowering date.

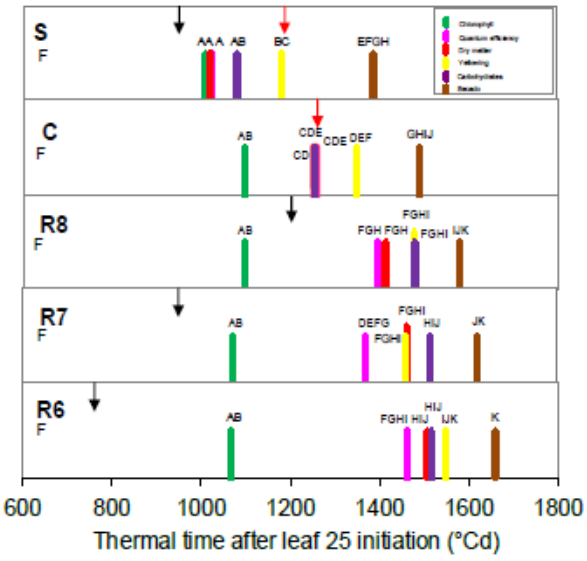

Fig. 4 Events related to leaf senescence sorted in a thermal time scale from the initiation of leaf 25 in the apex. Different letters indicate that 2 events from the same or a different treatment are significantly different (α=0.05). Arrows indicate treatment application (black) or physiological maturity (red). F indicates flowering date.

Effect of SSR on the sequence and the moment of occurrence of the events:

In leaf 15, chlorophyll, Qyield, dry matter and carbohydrates were the first events in the control. More than 150°Cd later happened yellowing ($p<0,05$), and then leaf definitively dead ($p<0,05$, Fig. 2, C). SSR treatments did not affect neither the sequence nor the moment of occurrence of the events, in comparison with the control ($p>0,05$, Fig.2 R7, R8 y S), excepted R6 where Qyield was delayed ($p<0,05$, Fig. 2, R6).

In leaf 20, chlorophyll, Qyield and dry matter were the first events occurred in C treatment ($p<0.05$). Then, 100°Cd and 200°Cd later were observed yellowing and carbohydrates ($p<0.05$), respectively, finally dying at 1500°Cd after its initiation ($p<0.05$, Fig. 3 C). In R6, a delay in all events was observed with exception of chlorophyll (Fig. 3 R6). Qyield and dry matter occurred almost 400°Cd later than C, and yellowing, carbohydrates and death about 180°Cd later ($p<0.05$, Fig. 3 R6). In R7, dry matter and carbohydrates were delayed near 200°Cd from C ($p<0.05$, Fig. 3 R7). The carbohydrates was the only event affected by R8 treatment (delayed 130°Cd in comparison with the C, $p<0.05$, Fig. 3 R8). Shading advanced 250°Cd the occurrence of carbohydrates and dead ($p<0.05$, Fig. 3 S). SSR treatments did not affect the sequence of events in leaf 20, according to our evaluation parameters (see materials and methods).

In leaf 25, chlorophyll was the first event in the control treatment. 160°Cd later ($p<0.05$), occurred Qyield, dry matter and carbohydrates. Near 100°Cd yellowing did not differ from the previous events ($p>0.05$), and finally leaf died 140°Cd later ($p<0.05$, Fig. 4 C). Treatment R6 delayed all the events, excepted chlorophyll, between 170°C and 250°C ($p<0.05$, Fig. 4 R6). When treatment was applied in R7 either dry matter or carbohydrates were delayed from the control ($p<0.05$, Fig. 4 R7). In R8, also Qyield was delayed ($p<0.05$, Fig. 4 R8). SSR decrease by shading, advanced all the studied events ($p<0.05$) save chlorophyll and yellowing, being dry matter and Qyield the events the plus affected (more than 230°Cd in comparison with C, $p<0.05$, Fig. 4 S). SSR treatments did not affect the sequence of events in leaf 25.

Effect of the leaf age on the sequence and the moment of occurrence of the events:

Life duration of leaf 15 was 184°Cd and 166°Cd shorter than those of leaf 20 and 25 (comparing the last event, $p<0.05$, Fig. 5). Sequence of events in leaf 15 and leaf 25 was similar. In leaf 20 carbohydrates occurred between yellowing and dead, in contrast with leaves 15 and 25 in which it occurred before yellowing ($p<0.05$, Fig. 5). In leaf 15, all the events occurred before than in leaves 20 and 25 ($p<0.05$). In this last, Qyield occurred 132°Cd later than in leaf 20 ($p<0.05$), while carbohydrates occurred 107°Cd before, even if difference was not significant ($p>0.05$, Fig. 5).

SSR effect on the events related to the senescence according to the time of the filling period:

The increase in SSR by removing the head at different times of the grain filling period lead to a delay in the occurrence of all events studied in this work except the fall of chlorophyll to 80% of its maximum value (Fig. 6.A, B, C, D, E and F). Depending on the event, the time at which the increase in SSR occurred, and the leaf, this delay reached 400°Cd (Fig. 6.B). Overall, the event lasted more when the time between the application of the treatment and the occurrence of the event was higher. It can be observed in certain events as the fall of the dry matter, yellowing and leaf dead, an approximately linear relationship between both variables (Fig. 6.C, D and F). In other events, such as the fall of carbohydrates, the delay was approximately constant at changes over 450°Cd in the period between the application of the treatment and the occurrence of the event (Fig. 6.E). Conversely to an increase in SSR, its reduction by applying a shade, advanced all the events studied in this work (Fig. 6.A, B, C, D, E and F). Depending on the event and the leaf, this advance ranged between 88°Cd (chlorophyll, Fig. 6.A) and 256°Cd (carbohydrates, Fig. 6.E).

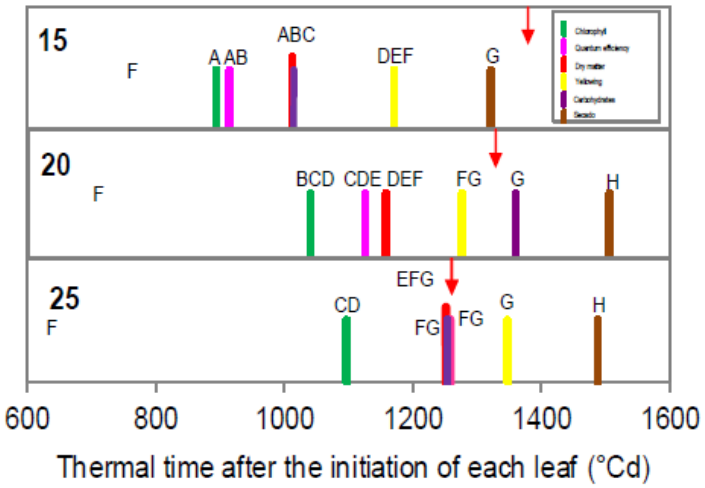

Fig. 5 Events related to leaf senescence in leaf 15, 20 and 25 of the control treatment sorted in a thermal time scale from the initiation of each leaf in the apex. Different letters indicate that 2 events from the same or a different leaf are significantly different (α=0.05). Red arrows indicate physiological maturity, and F the flowering date.

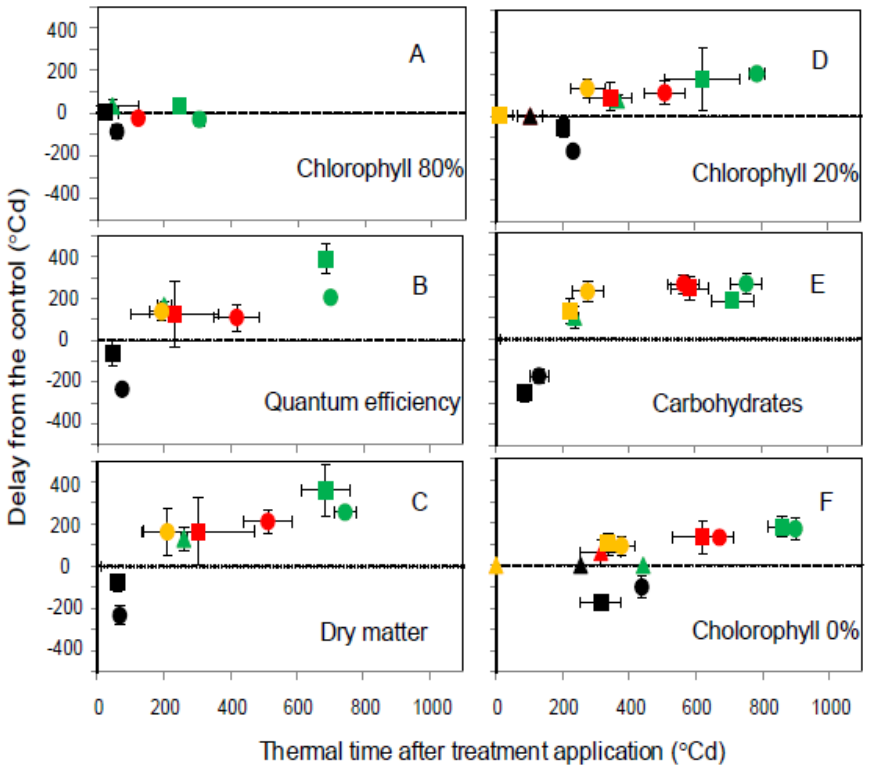

Fig. 6 Delay from the control of the events time of occurrence as a function of the time after the treatment application. Treatments: head removal at the developmental stages R6 (green), R7 (red) and R8 (orange), and shading (S, black). Leaves: 15 (triangle), 20 (square) and 25 (circle). Vertical and horizontal lines on the symbol represent the standard error of the mean value (n=9). Data from events which naturally occurred before treatment application was not included.

The period between treatment application and the occurrence of the events in leaves 20 and 25 was longer than in leaf 15 (Fig. 6.A, B, C, D, E y F). Even, in this last leaf some events occurred previously to the application of certain treatments, and therefore did not appear in the corresponding figures (Fig. 6.A, B, C, D y E). The delay or advance in the occurrence of events by effect of SSR increasing or decreasing, respectively, in the leaves 20 and 25, was generally similar excepted in Qyield and dry matter, where leaf 20 was affected more than 25, in treatment R6, and conversely, leaf 25 was more affected than leaf 20 in shading treatment (Fig. 6.B and C).

DISCUSSION

The increase in SSR by head removal delayed leaf senescence measured through all the variables used as possible senescence indicators in this study. This occurred in a greater or lesser extent in the 3 leaves studied and in spite of the time of the grain filling period in which occurred the increase in SSR (Fig. 1). Ho and Below (1989) and Purohit (1982) also observed a delay in leaf senescence after head removal by measuring chlorophyll content. Sadras et al. (2000) reported comparable results working also with leaf nitrogen and dry matter concentration. The absence of the reproductive sink in annuals prevents grain demand and consequently nutrient remobilization from leaves. This will allow keep longer cell structure and functionality. Otherwise, the lack of the head in sunflower was related to a prolongation of roots functionality, which was associated to leaf duration (Lisanti et al., 2012). Conversely, a decrease in SSR by shading produced an advancement of leaf senescence measured through all studied variables. The decrease of incident radiation causes a decrease in photosynthesis which could be the cause of the onset of senescence.

When we classed our events in a unique thermal time scale we established a sequence. Even if each event, as observed for the complete evolution of the respective variable, mostly delayed or advanced with an increase or a decrease in SSR, respectively, the sequence established remained irreversible not only to changes in SSR, but also to the moment of these changes (Fig 2, 3 and 4) and to the age of the three studied leaves (Fig 5). From these results we could assume that once triggered, leaf senescence would follow the same metabolic signaling pathway.

In other attempt to classify senescence events in sunflower, Moschen et al. (2012) observed that N content in leaf 25 occurred much earlier than our first event, the drop of chlorophyll to 80% of its maximal value. In our work, the first event was the only one remaining stable after changes in SSR and in leaf age, although in several cases (especially in leaf 15) this event occurred before the application of the treatment. From these results we could guess that changes in SSR did not prevent senescence triggering, but they shortened or lengthened senescence period.

As change in SSR was before, the effect on the occurrence of an event was mostly higher. The exceptions were chlorophyll drop to 80% (above mentioned) and carbohydrates drop (Fig. 6). This was probably related to integrating carbohydrates functions in senescence regulation (Wingler and Purdy, 2006).

CONCLUSION

this work demonstrates that SSR during the grain filling period can advance or delay leaf senescence in sunflower. This effect did not involve the onset but the subsequent evolution of senescence. The sequence of studied events related to senescence was not modified by changes in SSR. Senescence in lower leaves occurred earlier than in upper leaves but preserving the same sequence of events.

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TWO SIMPLE MODELS INCLUDING THE SOURCE/SINK RATIO TO EXPLAIN BLACK STEM BY *PHOMA MACDONALDII* IN SUNFLOWER

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ABSTRACT

Black stem (BS) by *Phoma macdonaldii* Boer of sunflower (*Helianthus annuus* L.) is the most prevalent foliar disease in the Buenos Aires province, main sunflower area of Argentina. The source- sink ratio (SSR) of sunflower crop affects the plant susceptibility to BS, although this effect may be influenced by several factors. The aim of this work was to establish simple models to take into account the SSR of sunflower to estimate BS incidence and severity, in different environmental conditions, hybrid cultivars and leaf stratums. Three field experiments, including two hybrids, were performed at Balcarce, Argentina. The SSR was modified by grain excision or shading, during the grain filling period. BS incidence and severity (in nodes 8, 12 and 20) were evaluated weekly from flowering. SSR took account of a significant fraction of the yearly incidence ($R2 \geq 0.619$) and severity variation ($R2 \geq 0.458$), both hybrids comprised. To include the annual variability, regression analyses were performed between meteorological and physiological variables (PAR, % interception, number of rainy days, mm of rainfall) and BS incidence and severity. In the case of severity, the age of the leaf was also included. Multiple linear and non-linear models were selected by the analysis of stepwise and residual methods. About 80% of the total variation in incidence and severity of BS due to hybrids, experiments and treatments, was explained by simple models including SSR and number of rainy days ($p \leq 0.0001$), or SSR, number of rainy days and leaf age ($p \leq 0.0001$), respectively. Simple models like these are potentially useful in the assistance to crop management, and could also be included to improve simulation models of diseases, growth and development in sunflower.

Key words: source- sink ratio, *Helianthus annuus* L., *Phoma macdonaldii* Boer., rainy days, age of the leaf

INTRODUCTION

The "black stem" (BS) caused by the necrotrophic fungus *Phoma macdonaldii* Boer (teleomorph *Leptosphaeria lindquistii*) is the most prevalent leaf disease in sunflower (*Helianthus annuus* L.) in the Buenos Aires province (Lazzaro *et al*., 2013), the main sunflower production area of Argentina (1.16 to 2.40 thousand ton of grains in the last ten years, SIIA-MAGPyA, 2015). Symptoms appear on the stem near flowering stage, progress from bottom to upper leaves, and are usually associated to previous necrosis in veins, petiole and/or leaf lamina (Bordat *et al*., 2011). *P. macdonaldii* also attacks roots and the collar of the plant, producing a stem girdling lesion at the soil level at the beginning of premature ripening (Donald *et al*., 1987). As yet, there are no reports of sunflower genotypes with high resistance to BS or premature ripening. Yield losses between 10 and 30 % were reported to be associated to BS (Debaeke and Pérès, 2003; Velásquez and Formento, 2003) or premature ripening (Carson, 1991) via a

decrease in intercepted radiation, related to premature leaf senescence and/or in radiation use efficiency (Quiroz *et al*., 2014).

Crop models have many current and potential uses for answering questions in research and crop management. Models can assist in synthesis of research understanding about the interactions of genetics, physiology, and the environment, integration across disciplines, and organization of data Boote *et al*., 1996. Often, mathematicians and statisticians models are used to study and describe plant growth, the effect of management practices and development of diseases Campbell y Madden, 1990 Hernandez *et al*., 2009.

BS estimate models have been developed by Debaeke and Peres (2003) and Desanlis (2013). These models consider epidemiological aspects as canopy microclimate (relative humidity and temperature), plant growth (leaf area index) and fungicide treatment, to account for climatic and agronomic limitations to fungus development. Further, in a previous work by our group were determined that BS incidence and severity were inversely related to the source -sink ratio (Nuñez Bordoy *et al*., 2012). These relations suggests the existence of a stem carbohydrates threshold under of this the BS incidence or severity start to increase. The carbohydrate content of the plant could closely relate to the existence of one or more substances that inhibit the growth of fungi, such as phenolics and terpenes (Silva Acuña *et al*., 2000) associated to plant response against pathogens. Because the relation between SSR and BS symptom could be affected by plant intrinsic factors or by meteorological and agronomic conditions we formulate the objective to establish simple models to take into account the SSR of sunflower to estimate BS incidence and severity, in different environmental conditions, hybrid cultivars and leaf stratums.

MATERIALS AND METHODS

Three field experiments (Exp. 1, Exp. 2 and Exp. 3) were the INTA Balcarce Experimental Station, Argentina (37º45' S, 58º18' W). Hybrids VDH 487 (Advanta Seeds SAIC, Argentina), 81 days from emergence to flowering, and Baqueano (KWS Argentina SA), 88 days from emergence to flowering, were sown on the Typic Argiudol soil (USDA taxonomy, organic matter 7.4%, P-Bray 25.8 ppm). Plant density was adjusted manually to 5.6 plants m-2 in the three experiments and rows were 0.7 m apart. The crops were grown under good water and nutrients conditions. Weeds and insects were controlled adequately through cultural and chemical techniques.

The experiments were conducted under conditions of natural inoculation of *P. macdonaldii* in plots infected with the pathogen (verified in previous experiments). Additionally, pieces of infected milled sunflower plants from previous experiments were homogeneously distributed in the plots during V6 stage (Schneiter and Miller, 1981) to assure *P. macdonaldii* presence.

In order to modify the source-sink ratio (SSR) with a different approach (modifying the sink or the source), two sorts of treatments were applied after the end of flowering (R6, Schneiter and Miller, 1981):

1. Grains excision: grains from about two (G↓) or three (G↓↓) quarters of the head were carefully removed (Echarte *et al*., 2012).

2. Reduction of solar radiation: a 38 % uniform shading with black, synthetic and neutral mesh cloth (S) was applied (Dosio *et al*; 2000).

3. An untreated plot was kept as control (C).

Source sink treatments and hybrids were combined in a randomized complete block design with three replicates. Each plot consisted of four rows of 6 m long, spaced at 0.7 m.

Daily global incident radiation and rainfall were measured in a weather station located 400 m from the experiments. Daily mean air temperature in treatments control and shaded (S) was also measured at leaves 8, 12, 20 and 28 level (from the bottom of the plant) with copper/constantan thermocouples. The

average of the temperature at these three levels was used for thermal time estimates. Data were averaged every 3600 s, and recorded by a data logger (Cavadevices.com, Buenos Aires, Argentina). Thermal time was calculated by daily integration of air temperature and a base of 6 °C (Kiniry *et al*. 1992), and cumulated from flowering.

Daily incident photosynthetically active radiation (PAR) was calculated as 0.48 × global daily incident radiation. The proportion of PAR intercepted by the crop at noon was determined according to Gallo and Daughtry (1986) as (1 − Rb/Ro), where Rb is PAR measured below the last green leaf and Ro is PAR measured above the canopy. Rb and Ro were measured weekly at solar noon (±1 h) with a line quantum sensor (LI-191SB, LI-COR, Lincoln, NE, USA). The daily proportion of PAR intercepted between two measurements was calculated by linear interpolation. The daily intercepted PAR (iPAR) was calculated as the product of the daily incident PAR and the daily proportion of PAR intercepted. The iPAR was cumulated from flowering to physiological maturity (PM).

Incidence of BS by *P. macdonaldii* (I %) was evaluated every 7-10 d in 3 plants per plot (n=9), as the ratio between the affected to the total number of nodes per plant. Severity of BS by *P. macdonaldii* (S %) was evaluated every 7-10 days in leaves 8, 12, 20 and 28 (from the bottom of the plant), selected to obtain a suitable plant profile of BS severity, in 3 plants per plot (n=9), following the methodology proposed in *Quiroz et al*. (2014). BS incidence and BS severity were estimated at 300, 350, 400 °Cd and PM by interpolation between two successive measurements.

The source–sink ratio (SSR) was periodically calculated during the grain filling period as the quotient of accumulated iPAR and the grain number per plant affected by the grain weight of treatment G↓↓, considered the closest to the potential weight. SSR was estimated at 300, 350, 400 °Cd and PM by interpolation between two successive measurements.

Data of SSR, incidence and severity of BS were processed by analysis of variance procedures (INFOSTAT Professional v.1.1, Di Rienzo *et al*., 2010). Differences among treatments means were evaluated with the LSD test ($P \leq 0.05$). Data of incidence and severity of BS as a function of SSR were adjusted to exponential models (Nuñez Bordoy *et al*., 2012) at 300, 350 and 400 °Cd from flowering and at PM (Sigma Plot v. 11.0 Systat Software Inc., 2010). For each variable the model with the greatest signification (α) and coefficient of determination (R2) was selected. Models did not include outliers.

To include the annual variability, regression analyses were performed between meteorological variables (PAR, % interception, number of rainy days, mm of rainfall) and BS incidence and severity. In the case of severity, the age of the leaf was also included. Multiple linear and non-linear models were done by the analysis of stepwise and residual methods (INFOSTAT Professional v.1.1, Di Rienzo *et al*., 2010).

RESULTS AND DISCUSSION

Source-sink ratio after flowering slightly increased or remained steady in plants from control and shading treatments, while grain excision (G↓ and G↓↓) mainly increased it in all tested situations (Fig. 1). SSR increase by grain removal is attributable to a higher retention of green leaves which still intercept solar radiation (data not shown), inclusive in some cases, SSR still increases yet after physiological maturity. Although productive sense of SSR after the end of grain filling is questionable, if photosynthesis is yet detectable, assimilates could be stocked in alternative sinks like stem.

An interaction between the effect of the experiment and that of the treatment ($p<0.0044$) was observed for SSR at 400ºCd after flowering (Fig. 1.A, B, C and D). No differences between S and control treatments were observed in Exp.1 and Exp.2 ($p>0.05$), probably because of a mild drop in the grain number (data not shown). Since grain number in sunflower is finally set near 20 days after flowering (Connor and Hall, 1997), in some cases we probably applied shading treatment just before grain number was set. Grain excision treatments showed a higher SSR in Exp.1 (216%, G↓↓) and Exp.2 (47% and 200%, G↓ and G↓↓, respectively) than that of the control (Fig. 1.A, B, C and D). Furthermore, hybrid Baqueano had SSR

more than 30% higher than hybrid VDH 487 at this thermal time after flowering (p<0.0001, Fig. 1.A, B, C and D), due to a lesser demand from grains with a small potential weight than hybrid VDH 487.

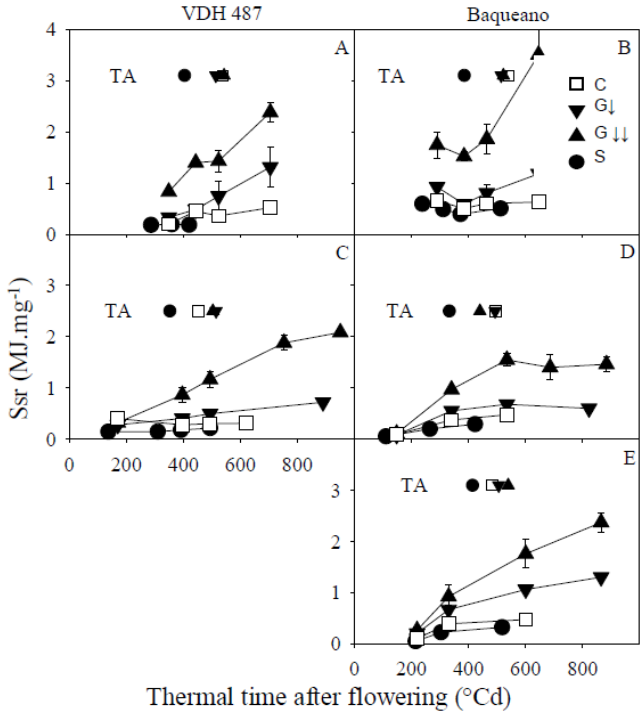

Fig. 1. Source-sink ratio (SSR) as a function of the thermal time after flowering for hybrids VDH 487 (A and C) and Baqueano (B, D and E), in Exp.1 (A and B), Exp. 2 (C and D) and Exp.3 (E). Treatments: grain excision from about two (G↓, inverted triangles), or three (G↓↓, triangles) quarters of the head, shading during the filling period (S, solid circles) and control (C, open squares). Treatment application (TA) and physiological maturity for each treatment are indicated at the top of the chart. Vertical bars on the symbols indicate the standard error of the mean value (n=9).

Incidence of BS by *P. macdonaldii* increased during the grain filling period in all treatments, hybrids and experiments (Fig. 2). Shading treatment almost always accelerated the incidence increase (Fig. 2.A, B, C and D), while grain excision usually reduced it (Fig. 2.A, C, D and E). BS incidence at 400ºCd after flowering was highly affected by applied treatments (p<0.0001, Fig. 2 A, B, C and D). Plants from shading treatment increased 42% BS incidence (p<0.05), while those from the higher grain excision treatment (G↓↓) decreased it 16%, in comparison with control plants (p<0.05, Fig. 2.A, B, C and D). No hybrid effect was observed for this variable (p=0.4732). The lower values of BS incidence were observed in Exp. 3, while the highest in Exp. 1 (14% and 59%, respectively, mean of all applied treatments, Fig. 2.B, D and E). An interaction between the treatment and the experiment was observed for this variable (p<0.0001).

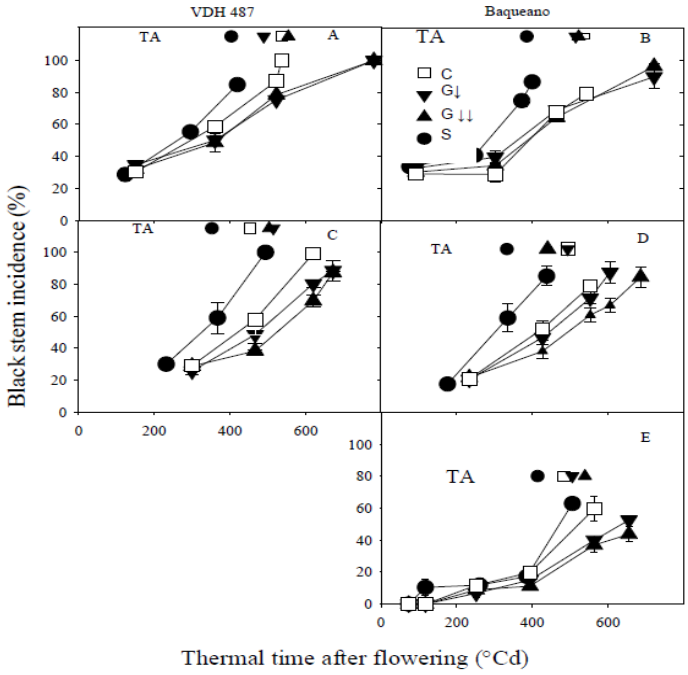

Fig. 2. Black stem incidence (%) as a function of the thermal time after flowering for hybrids VDH 487 (A and C), Baqueano (B, D and E) in Exp.1 (A and B), Exp. 2 (C and D) and Exp.3 (E). Treatments: grain excision from about two (G↓, inverted triangles), or three (G↓↓, triangles) quarters of the head, shading during the filling period (S, solid circles) and control (C, open squares). Treatment application (TA) and physiological maturity for each treatment are indicated at the top of the chart. Vertical bars on the symbols indicate the standard error of the mean value (n=9).

Symptoms of BS by *P. macdonaldii* appeared first in leaves from the bottom of the plant and progressed upwards to upper leaves in all hybrids, treatments and experiments (p<0.0001). This result corroborates the rise acropetal nature of this disease (Quiroz, *et al.* 2014). In turn, leaf functionality is associated to incident light and therefore to its position on the stem. Bottom leaves receive an intensity and aquality of light (red/far red ratio) poorer than upper ones which make them senesce before (Rousseaux *et al*., 1996), may be more susceptible to *P. macdonaldii* infections for photosynthetic stress-translocation balance.

The onset and the progress on the node of BS severity symptoms were advanced in shading treatment (S) and delayed in grain excision treatments (G↓, G↓↓) in most hybrids and experiments (Fig. 3, leaf 20 as example).

A significant effect of the treatments (p<0.0001), the hybrid (p=0.0074) and the leaf (p<0.0001) on BS severity was observed at 400ºCd after flowering. Shading increased 50% BS severity while grain excision reduced it 18% and 33% (treatments G↓ and G↓↓, respectively, p<0.05) in comparison with the control (Fig. 3.A, B, C and D, leaf 20 as example). Hybrid VDH 487 showed severity symptoms more than 10% higher than hybrid Baqueano (p<0.05). Leaf 8 was the most affected by BS severity, followed by leaf 12 and leaf 20 which was the less affected (difference of 80% between leaves 8 and 20, p<0.05).

The effect of the experiment interacted with those of the treatment and the leaf (p=0.0033 and p=0.0025, respectively) Treatment S during Exp. 1 and Exp. 2 presented higher BS severity values than the rest of the treatments at 400ºCd after flowering (p<0.05). In Exp. 3, we observed the lesser BS severity in all treatments (Fig. 3. B, D and E, leaf 20 as example). Leaves 8, 12 and 20 presented lower BS severity values in Exp. 3 in comparison with Exp. 1 and Exp. 2 (p<0.05, Fig. 3. B, D and E, leaf 20 as example). There were not observed symptoms of BS severity in leaf 28 at 400ºCd after flowering (data not shown).

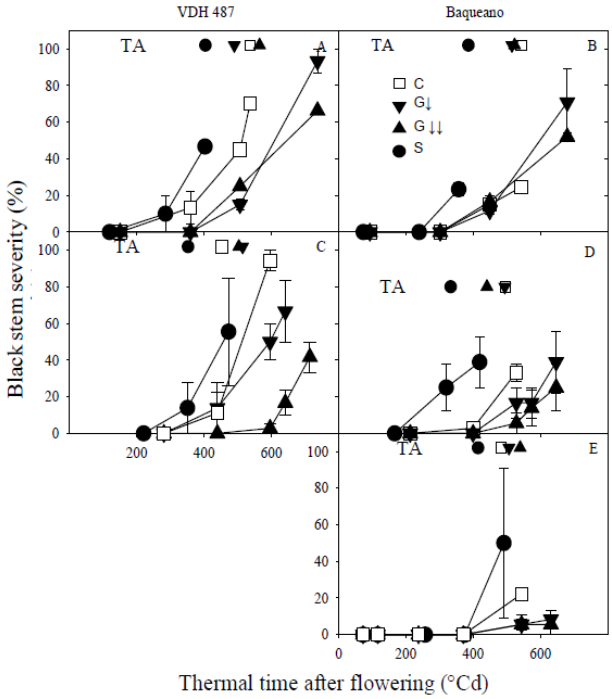

Fig. 3. Black stem severity (%) in leaf 20 as a function of the thermal time after flowering for hybrids VDH 487 (A and C), Baqueano (B, D and E), in Exp.1 (A and B), Exp. 2 (C and D) and Exp.3 (E). Treatments: grain excision from about two (G↓, inverted triangles), or three (G↓↓, triangles) quarters of the head, shading during the filling period (S, solid circles) and control (C, open squares). Treatment application (TA) and physiological maturity for each treatment are indicated at the top of the chart. Vertical bars on the symbols indicate the standard error of the mean value (n=9).

As stated in MM, results of incidence and severity of BS as a function of SSR were adjusted to exponential models at 300°Cd, 350°Cd and 400°Cd from flowering and at physiological maturity, however we decided to carry out modeling with results from 400°Cd from flowering for three reasons: (i) before this date, disease symptoms were not important yet (Fig. 2 and Fig. 3), (ii) this was the very last date in which we kept all the treatments for the analysis, since in shading treatment, the stem of the plants became black, impeding measurements, and (iii) adjustments at 300°Cd, 350°Cd and 400°Cd from flowering and at physiological maturity presented lower significance and/or determination coefficient (data not shown). Both incidence and severity of BS by *P. macdonaldii* decreased with increasing SSR. As a consequence of the highly significant effect of the experiment and the leaf presented above, results from treatments at 400°Cd from flowering adjusted to negative exponential models for each experiment in the case of BS incidence ($R2 \geq 0.619$, Fig. 4.A), and for each measured leaf in the case of BS severity ($R2 \geq 0.458$, Fig. 4.B).

Similar results were reported by Eslava *et al.* (2007) observed root and presence of mycelia from *Fusarium spp*. on the stem base. This function suggests the existence of a stem carbohydrates threshold under of this the BS severity start to increase, as Davet and Serieys (1987) had shown for *Macrophomina phaseolina* infection at base stem in sunflower.

The higher BS incidence was observed in Exp. 1 (Fig. 4.A), while the higher BS severity was almost always observed on leaf 8 and the lower on leaf 20 (Fig. 4.B). Increasing SSR from 0.1 to 0.6 MJ.mg-1 was associated to a decrease in BS incidence up to near 40% of the maximum incidence observed (Exp. 2, Fig. 4.A), and 70% of the maximum severity observed (leaf 20, Fig. 4.B). Values of SSR higher than 0.6 MJ.mg-1 did not affect significantly, neither incidence, nor severity of BS (Fig. 4.A and B).

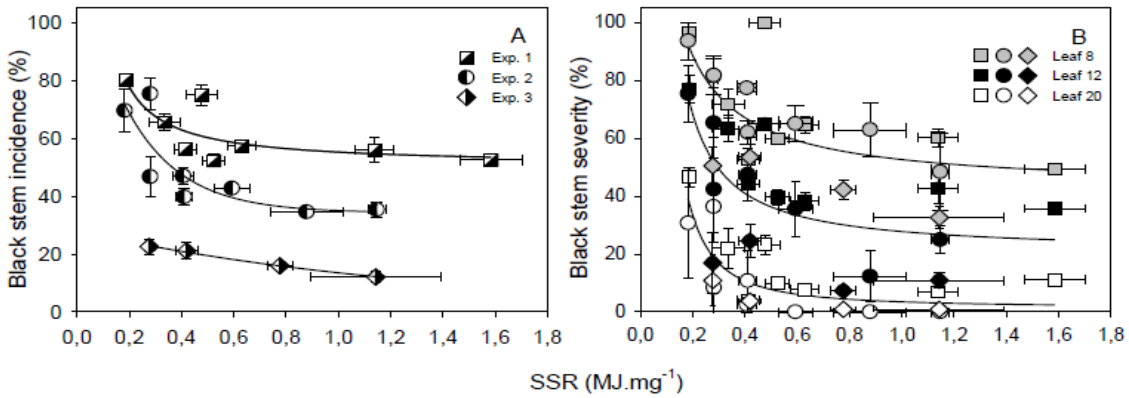

Fig. 4. Incidence (A) and severity (B) of black stem by *P. macdonaldii* as a function of the source-sink ratio (SSR, MJ.mg-1) at 400°Cd after flowering in Exp. 1 (squares), Exp. 2 (circles) and Exp. 3 (diamonds). Symbols filled in combined black/white indicate BS incidence (A). BS severity is indicate with grey (leaf 8), black (leaf 12) or white (leaf 20) symbols (B). Shading value from Exp. 1 in hybrid Baqueano corresponded to 372Cd after flowering. Curvilinear lines illustrate the adjustment of the results to the model: *BSincidence(%)=50.524*exp(0.089/(SSR+0.007)), p=0.0895, R2=0.619, n=8, for Exp.1; BSincidence(%)=34.216+89.563*exp(-4.774*SSR), p=0.05, R2=0.698, n=8, for Exp.2, and BSincidence(%)=27.991*exp(-0.730*SSR), p=0.0025, R2=0.995, n=4, for Exp.3. BSseverity(%)=48.051+70.593*exp(-3.066*SSR), p=0.0053, R2=0.46, for leaf 8; BSseverity(%)=24.721+97.108*exp(-4.186*SSR), p=0.0055, R2=0.458, for leaf 12, and BSseverity(%)=3.962+146.129*exp(-7.9*SSR), p=0.0002, R2=0.63,* for leaf 20.Vertical and horizontal bars on the symbols indicate the standard error of the mean value of BS incidence or severity and SSR, respectively (n=9).

After a multiple regression analysis including, SSR, the number of rainy days, the photosynthetically active radiation (PAR), the % of interception of radiation, the mm of rainfall and the age of the leaf, the models: BS incidence = -26.78+7.56*number of rainy days+56.52*exp(-4.138*SSR), s.e.=11.97%, and BS severity = -374+0.26*age of the leaf+10.96*number of rainy days+95.067*exp(-5.033*SSR), s.e.=12.15%, explained about 80% of the variability in BS incidence and BS severity, in an estimated/observed plot (Fig. 5.A and B).

While in the case of BS incidence a linear model setting between estimated and observed values did not differ from the 1:1 bisector (p=0.17), in BS severity, both severity intercept (p=0.0006) and the slope (p=0.0004) differed from "0" and "1", respectively. The observed values of BS severity were slightly underestimated or overestimated by the model in low and high ranges of the scale (0-20% and 80-100%, respectively, Fig. 5.A and B). Nevertheless, the magnitude of these differences was lower than the s.e. of the model.

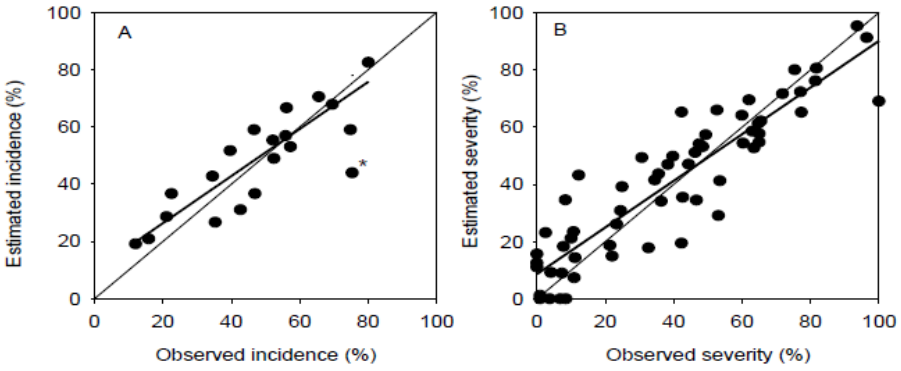

Fig. 5. Observed and estimated values of BS incidence (A) and BS severity (B) obtained from the field experiments and from the models BS incidence=-26.78+7.56*number of rainy days+56.52*exp(-4.138*SSR), s.e.=11.97%, and BS severity=-374+0.26*age of the leaf+10.96*number of rainy days+95.067*exp(-5.033*SSR), s.e.=12.15%, respectively. The thick lines result from the adjustment of the results to linear models (estimated incidence=9.909+0.822*observed incidence; R2=0.798; p<0.0001; n=19, and estimated severity=8.625+0.815*observed severity; R2=0.825; p <0.0001; n=60). The asterisk near a symbol indicates that this result was an outlier (estimated - observed > 2.5 standard deviation), and was not consider in the adjustment. The thin line represents the 1:1 values.

The function between SSR and BS symptom was strongly affected by disease variable (incidence or severity), the experimental years and by number of nude. In concordance with Quiroz (2015), the number of rainy days from flowering to 400ºCd after flowering was the meteorological variable which better explained the effect of the experiment on BS incidence.

Rainfall events were the principal epidemiological indicator affecting diseases expression in the relationship SSR vs BS. In agreement, Délos *et al.* (1997) demonstrated that rainfall correlated with ascospores release from the primary source of inoculum (stubble, seeds, etc.), and not with inoculum amount (Descops *et al*., 2012).

The age of the leaf (in ºCd from its appearance on the apex) was the variable which better explained differences observed among leaves. Time passed between *P. macdonaldii* inoculation in petiole and symptons appearance in stem, depend on phenological stage (related to leaf age) and the cultivar (Larfeil *et al*., 2010), exhibiting later stages the shortest period. Leaf age affects photosynthesis, being older leaves less efficient than younger ones (English *et al*., 1979) resulting in early senescence and probably with a higher susceptible to *P. macdonaldii* infections.

Our models were constructed without water and nutrients limitation during three years and with two hybrids. We considered ecophysiological and epimediological interactions from

the crop and disease during the grain filling period. Other empiric predictive models, developed including a great range of crop management and environments (Debaeke and Péres, 2003), established a positive relationship between LAI or iPAR at flowering and BS and postulate that the more favourable microclimate of dense stands canopies could explain the higher level of infection of *P. macdonaldii*. Later, Desanlis (2013) deepened this epimediological approach proposing a conceptual model where the potential infection rate could be reduced by several reduction factors (RF): microclimate (HR, T), plant growth (LAI) and fungicide treatment, to account for climatic and agronomic limitations to fungus development.

CONCLUSION

The estimate BS models proposed in this paper include a new concept relating SSR, as a crop ecophysiological condition, to the plant susceptibility to *P. macdonaldii* infection. In turn, these models can contemplate different environmental conditions, hybrid cultivars and leaf stratums. This approach could be combined with the other mentioned models. For this it would be necessary to broaden the crop managements (according to Debaeke y Peres 2013) and stressed environments (e.g., crop damage, water or nutrient deficits, etc.).

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CALLUS FORMATION AND PLANT REGENERATION IN SUNFLOWER (*HELIANTHUS* L., ASTERACEAE) IN VITRO TISSUE CULTURE

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ABSTRACT

For the sake of introduction of sunflower to *in vitro* culture the seeds and flowers of different wild species were used: *Helianthus annuus* (three samples), *H. decapetalus*, *H. giganteus*, *H. macrophyllus*, *H. nutalli*, *H. occidentalis* ssp. occidentalis, *H. tuberosus* (four samples), *H. tuberosus* velmoren, and also cultivated variety "Master", lines VIR 114B (fertile) and VIR 114Apet (CMS). Fifteen combinations of plant growth regulators have been used. We were successful in introduction into the culture in vitro and obtain stably growing callus of *H. annuus* wild type (1 sample), *H. giganteus*, *H. occidentalis* ssp. occidentalis, *H. tuberosus* (callus from seeds and from flowers) and sunflower cv. "Master". The optimal medium for callus formation was MS with BAP and NAA (ratio 2:1) or TDZ. We could obtain successful regeneration from callus only for *H. giganteus* on MS media with BAP and NAA (ratio 2:1 or 5:1 in low concentration) when cultivated in the dark.

Key words: Sunflower, *In vitro* culture, Plant regeneration, *Helianthus giganteus*

Abbreviations: BAP - benzylaminopurine, NAA - naphthylacetic acid, TDZ - thidiazuron, AS - adenine sulfate

INTRODUCTION

Different methods for callus induction, adventitious bud formation, shoot multiplication and rooting of *in vitro* formed shoots of sunflower are described (Lupi et al., 1987; Weber et al., 2000; Rath and Pearson, 2004; Ozyigit et al, 2007; Neskorodov, 2011; Sujatha et al, 2012; Khalil et al., 2015 and others). According to the literature it is known that sunflower *in vitro* culture is quite successful, but stable plant regeneration from callus is difficult. The greatest success is achieved using direct regeneration from seed or immature embryos. But the perennial species and interspecific hybrids have very low seed production, or complete sterility (Gavrilova, Anisimova, 2003), so it is difficult to obtain enough amounts of seeds from them. In addition, regeneration by direct somatic embryogenesis (embryoidogenesis) is not suitable for use in genetic transformation protocol (Weber et al., 2000). The second difficulty for finding the optimal protocol of regeneration is a high variability in the response to the conditions of cultivation of species and varieties of sunflower (Ozyigit et al, 2007; Sujatha et al, 2012).

In sunflower investigation the obtaining of a new ways of plants regeneration from callus cultures *in vitro* is of current importance. It is necessary, using different kinds and types of sunflower explants to achieve the production of a stable callus culture, and then to choose the optimal conditions for successful regeneration of plants from callus.

MATERIALS AND METHODS

Helianthus annuus wild type (three different samples), *H. decapetalus*, *H. giganteus*, *H. macrophyllus*, *H. nutalli*, *H. occidentalis* ssp. occidentalis, *H. tuberosus*, *H. tuberosus* velmoren, and also cultivated sunflower *H. annuus* variety "Master", lines VIR 114 B (fertile) and VIR 114 A pet (CMS) were chosen for the study. Seeds were collected from plants grown in the Kuban Experimental Station of VIR.

In most of the experiments we used seedlings obtained from the seeds as primary explants. In addition we used as explants the Jerusalem artichoke (*H. tuberosus*) flowers. Jerusalem artichoke has been chosen as a model, as the most accessible in our region (St. Petersburg and the Leningrad region) perennial sunflower species. Jerusalem artichoke inflorescences at the different flowering stages were collected from the three different samples.

In the first series of experiments, the seeds were superficially sterilized under complete aseptic conditions by soaking in 96% ethanol for 20 min., and then treated over the burner flame for 10-15 seconds. Seeds were germinated for 24 hr on filter paper moistened with distilled water. If at first day of germination the appearance of the hyphae of fungi in the testa was observed, the embryos of these seeds were extracted and subjected to additional treatment of 10% hydrogen peroxide solution in the course of 20 min., then washed twice with sterile distilled water.

Plantlets were transferred to Petri dishes on a ½MS medium (Murashige and Skoog (1962) medium with a half dose of nutrients). Two week later, plantlets were cut into pieces. As primary explants we used all parts of the plantlets: pieces of root, stem, cotyledon, and bud with the leaves. Pieces of plantlets were placed on the MS medium. Three modifications were used, depending on the added growth regulators (MS-1, MS-2, MS-3 - see table). The plates were cultivated at t + 22°C in the dark or light. Then callus was transplanted to five variants of culture medium (MS-4, MS-5, MS-6, MS-7, MS-8). The plates were cultivated at t + 26°C in the dark or under 16 hour photoperiod.

Table. The composition of growth factors added to the culture medium

Growth factors (mg/l)	MS-0	MS-1	MS-2	MS-3	MS-4	MS-5	MS-6	MS-7	MS-8	MS-9	MS-10	MS-11	MS-12	MS-13	MS-14	MS-15
BAP		2		0,1		2	4	4	4	0,2	0,5	1	1	0,5	1	
TDZ			2		2	1	1	1	1							
NAA		1		0,1		0,5	1	1,5	2	0,1	0,1	0,5	0,02		1	0,5
AS				40	40											

In the second series of experiments the tubular flowers were used from the inflorescence of Jerusalem artichoke as explants. The inflorescences were sterilized during 20 min. in 10% solution of hydrogen peroxide, and then transferred to distilled water, disassembled into individual flowers, which were placed for 15 min. in peroxide solution, then washed twice with sterile distilled water. We planted them on five variants of MS medium (MS-4, MS-5, MS-6, MS-7, MS-8).

For plant regeneration, the calluses derived both from seedlings and from flowers were transplanted to a new variant of culture medium (MS-9, MS-10, MS-11 and MS-12). The plates were cultivated at t + 26°C in the dark condition or under 16 hour photoperiod.

Callus with regeneration zones was transferred into a new version of the medium (MS-13, MS-14 and MS-15) to support the growth and rooting. Then, plants both having roots and without them were transplanted into larger flasks on medium without hormones (MS-0).

Subsequently, the plants were transferred from the sterile culture into non-sterile conditions. They were transplanted to pots filled with sand and placed in a climatic chamber at t= + 26°C under 16 hour photoperiod.

RESULTS AND DISCUSSIONS

We had introduced into the culture *in vitro* and obtained stably growing calluses of *H. annuus* wild type (1 sample), *H. giganteus*, *H. occidentalis* ssp. occidentalis, *H. tuberosus* (callus from seeds and from flowers) and *H. annuus* cv. "Master" (Fig. 1a,b,c: 2a,b).

We are faced with the fact that it is difficult to obtain sterile seedlings using freshly collected seeds. These seeds germinated worse and were poorly sterilized. Frequently the re-treatment of emerged embryos was necessary and so we released them from the seed coat, which was struck by mildew, and sterilized again.

To obtain a stable callus cultures from the seeds the following protocol was the most productive: 1) sterilization of seeds, 2) germination on filter paper moistened with distilled water, 3) re-sterilization and transfer of seedlings on a medium ½MS, 4) using all parts of the seedling (root, leaf, stem, cotyledon) as explants, 5) culturing in the dark condition or under 16 hour photoperiod (the second is preferable) and 6) regular transplanting every 6-8 weeks (Fig. 1c,d).

We observed intensive formation of a new callus on MS-1, MS-2, MS-3 or MS-11 media with equal success. So, callus was formed well on media containing NAA and BAR in the ratio 2:1 or in equal low concentration (0,1 mg/l) with AS, as well as on media with TDZ.

The appearance of callus formation centers was noted 7-10 days after transfering. Intensive callus formation continues from 2 to 2,5 months.

Callus formation was observed in different parts of seedling (from pieces, cotyledon, stem, leaves, roots, buds) (Fig. 1d,e).Cytology showed that in callus histogenesis, the formation of centers of meristematic activity and elements of the vascular system occur (Fig. 2h).

In the case of using the tubular flowers as a primary explants the optimal procedure for obtaining a callus is shorter: 1) surface sterilization of inflorescences, then isolation and sterilization of flowers, 2) culturing either in the darkness or under light and 3) regular transfers every 6-8 weeks.

We used for callus induction the five variants of culture medium (MS-4, MS-5, MS-6, MS-7, MS-8). Of these, only a MS-4 medium was bad for callus formation.

Within 7-14 days after the transfer of flowers on a medium, we observed changes similar to flowering in the wild - the disclosure of the tops in anthers (but pollen did not fall out, in contrast to the natural condition). 4-6 weeks later, the beginning of callus formation is noted which occurs intensively within 2,5-3 months, then the callus growth stops (Fig. 2c,d,e).

Callus initiation was observed at the bottom of the disc nectar, and along the upper edge of the tube sepals from anther and stigma tissues. Cytological study showed that in these parts the sections of tissue with small meristem-like cells with dense cytoplasm and nucleus are still persist, while the surrounding tissue consists of large vacuolated cells with badly viewed nucleus (Fig. 2f,g).

We can get the successful regeneration from callus only for *H. giganteus* on MS media with BAP and NAA (ratio 2:1 or 5:1 in low concentration) when cultivated in the darkness. More than one year passed since the seeds have been planted in a Petri dishes and callus was transplanted several times into new versions of the nutrient medium. In the medium MS-9 there were observed gemmo- and rhyzogenesis (shoot and root formation) and rhyzogenesis (root formation), on media MS-10 and MS-11 only gemmogenesis (shoot formation) (Fig. 1f,g,h).

In general, media supplemented with BAP and NAA in ratio 2:1 had a higher callus formation (MS-1, MS-11) as well as media with TDZ (MS-2) and with AS plus low concentration of BAP and NAA (MS-3).

Interestingly, the same range of concentration of 1,0mg/l BAP combined with 0,5mg/l NAA was reported by other researchers to give the highest shoot regeneration (Knittel et al., 1991; Baker et al., 1999; Rath and Pearson, 2004; Ozyigit et al., 2007; Khalil et al., 2015). Some of them noted the

importance of auxins/cytokinin balance for sunflower regeneration, but in our experiments for most samples of sunflower on media with BAP/NAA in such concentration we could get only callusogenesis.

In general it is worth paying attention to presence in cultural medium BAP and NAA in 2:1 ratio, as the most interesting in respect of stimulation of processes of a morphogenesis (callusogenesis, histogenesis, gemmogenesis, rhyzogenesis and a gemmorhyzogenesis) in sunflower culture *in vitro*.

Thus, in literature the different successful protocols which marked for this or that genotype of sunflower are noted, but there is no uniform scheme guaranteeing the success of micropropagation of any investigated sunflower varieties. The response to influence of growth factors depends strongly on the genotype chosen for experiment and is not predictable in advance. Also, the result depends on the choice of the explants and culture conditions (see review in Khalil et al., 2015). Here you can pay attention to more studied culture – corn for which the genes responsible for regeneration *in vitro* are already allocated (Tomes and Smith, 1985; Armstrong et al., 1992; Checheneva and Trukhanov, 1994 and others). Probably, sunflower has similar genes responsible for ability of cells to differentiate on the way of gemmo- or embryoidogenesis *in vitro*. There are works where the positive influence of alleles of several genes to *in vitro* morphogenesis of sunflower is found (Kostina et al., 2013, 2015).

Figure 1. Callus formation and regeneration of plant from seeds. ***a*** – *H. giganteus* plants and flower, ***b*** – *H. annuus* cv. Master, ***c*** – seedling in vitro, ***d*** – different parts of seedling in vitro, ***e*** – callus formation and rhyzogenesis in primary culture after 4 months, ***f*** – gemmogenesis after transferring on new medium, ***g*** – callus with shoots and roots after one year, ***h*** – new plant of *H. giganteus* in the pot. Scale bar – 5 mm

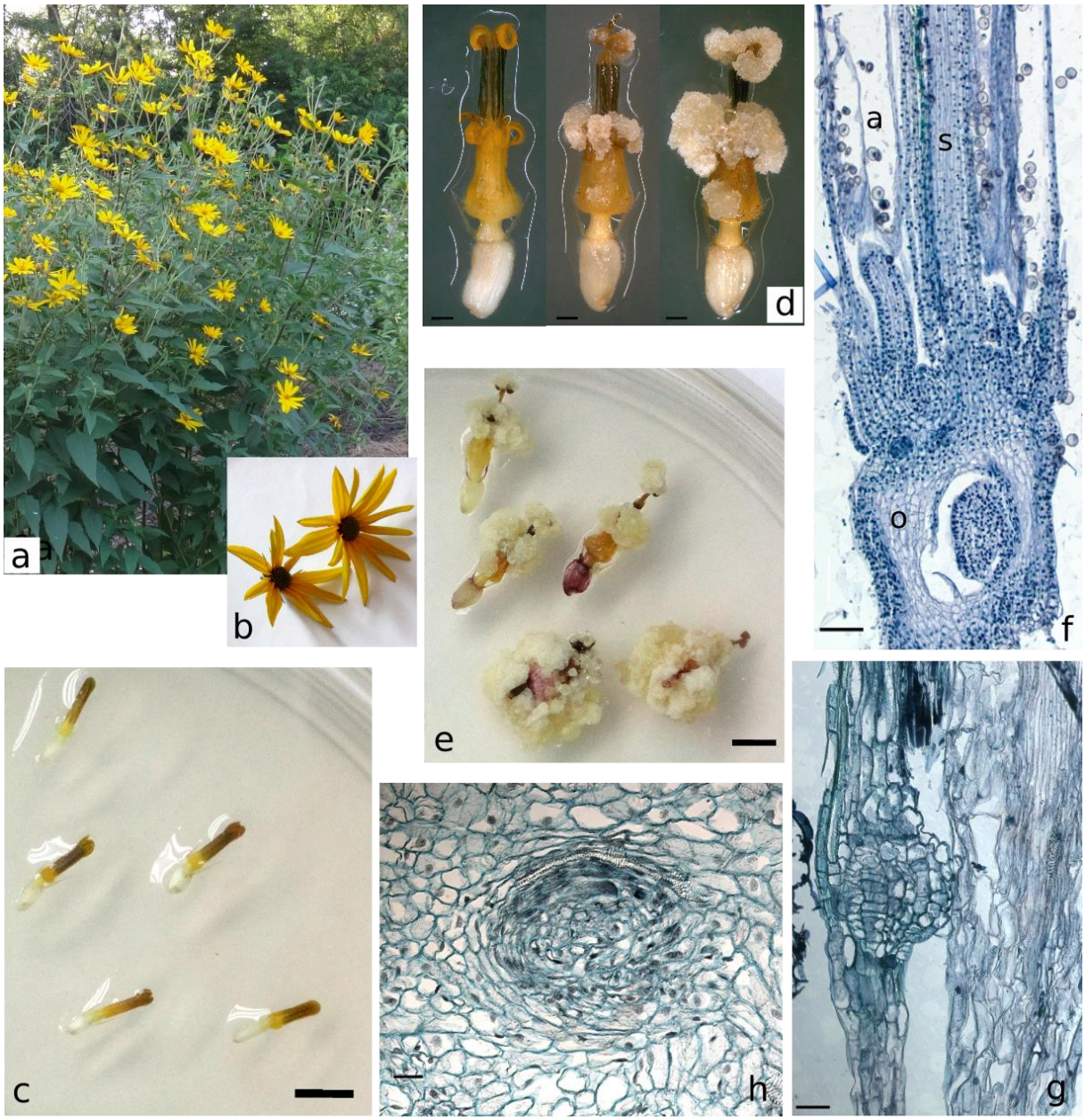

Figure 2. Callus formation from tubular flowers. ***a, b*** – *H. tuberosus* plants and flower, ***c*** – tubular flowers placed on Petri dishes in vitro, ***d*** – three different stage of callusogenesis, ***e*** – callus on tubular flowers after 2 months, ***f*** – longitudinal section of tubular flower (*a* – anther with pollen grain, *o* – ovary, *s* - stigma), ***g*** – zone of callus formation on anther stalk, ***h*** – formation of elements of vascular system in callus.
Scale bar: c, d, e – 5 mm, f – 100 mμ, g, h – 50 mμ.

ACKNOWLEDGEMENTS

The present study was carried out within the framework of the institutional research project (no. 01201255606) of the Komarov Botanical Institute of the Russian Academy of Sciences.

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OBSERVATIONS ON IMI GROUP HERBICIDES STRESS ON SUNFLOWER LEAVES (*HELIANTHUS ANNUUS* L.) BY SCANNING ELECTRON MICROSCOPY

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ABSTRACT

In this study, IMI group herbicides that used in sunflower cultivation and some sunflower cultivars that have resistance to these herbicides in different levels were used. Effects of herbicide application in different doses on surface of sunflower leaves were observed with light microscope and SEM. Seeds taken from Trakya Agricultural Research Institute was used as a material. Four different sunflower cultivars named SN:8 which is unresisting to IMI and SN:9, SN:10, SN:14 which are resistance to IMI were used in this study. Seed were germinated under controlled conditions in climate chamber and then seedlings were transferred to experimental parcels. Three different doses (125, 250, 375 ml/da) of herbicide were applied to seedlings that have 4-6 leaves. Leaf samples were collected on seventh day of herbicide treatment and observed under the light microscope. Fresh leaves obtained from the plant were used for SEM observations at Trakya University TUTAGEM laboratories. Anatomical investigations showed that there are differences on leaves surface between unresisting and resistance cultivars depending on different herbicide doses. In SEM analysis of the leaves, differences were seen in the number, size and structure of hairs and stoma cells.

Key Words : *Helianthus annuss* L., IMI, Leaves, Anatomy, SEM

A STUDY ON THE STANDARD GERMINATION AND SEEDLING GROWTH OF SOME CONFECTIONARY AND OIL SEED SUNFLOWER(*HELIANTHUS ANNUUS* L.) CULTIVARS

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ABSTRACT

It is very important to know sowing performance for production of sunflower that has wide economic importance. It is very common to observe variation in performance of same plant, the same cultivar seeds, even if the they have same chronological age class during their field propagation and performance. Performance difference among seed lots is due to variation in seed vigor. This study was carried out in the laboratory of the "Variety Registration and Seed Certification Center" between 15 January – 15 April 2014 to determine germination and seedling growth of some confectionary (cv. Palancı 1, Çiğdem 1) and oilseed sunflower cultivars namely (İnegöl Alası and Tunca, Sanbro and 08 TR 003) cultivars. In the study, standard germination tests measuring germination speed and germination vigor, seedling length, root length, above-ground wet and dry weight, below-ground wet and dry weight values were measured. According to the germination speed and germination vigor values, the oilseed cv. Tunca of sunflower that had the highest values of 96,50% and 98%, and confectionary type cv. Çiğdem1 had the lowest value of 77 % for germination speed and lowest value of 86% in germination vigor in terms of the general average for all cultivars.

Key Words: Sunflower (*Helianthus annuus* L.), Germination Speed, Germination Vigor, Seedling Length, Root Length.

INTRODUCTION

The oilseed vegetative groups cultivated in Turkey include sunflower, soybean, sesame, peanut, poppy, canola, safflower, cottonseed. 46 % from our vegetable oil production are meeting from sunflower (Anonym, 2007).Sunflower oil is one of vegetable oils with the highest nutritional value due to the containing high rate of unsaturated fatty acids (69%). Furthermore sunflower is also consumed as confectionary and 2,6 % of the production is for confectionary use. (Arıoğlu, 2000). Sunflower is also an important plant in terms of healthy nutrition. Sunflower seed which is also rich in terms of potassium and vitamin E is an important source of linoleic acid(Anonyms, 2004).

First stage at plant production is sowing of seed and germination. Good germination and ground output constitutes one of the most important stages of the plant productivity. During germination stage, the plant seeds having a difficult and uneven germination result in a heterogeneous output in cultivation environments and become source of significant losses both in terms of production and seed yield. Weeds, diseases and pests appearing together with irregular and late germination slow the growth of plants and have negative effects to productivity and the quality (Muhyaddin and Wiebe 1989). Germination; is the period providing the transition from the resting phase of seed to forming the plant and continuing till output of the radical-rootlets from the seed (Eser et al 2005). Conditions such as low and high soil temperature, thick seed coat, upper crust of the soil, heavy textured soils, soil salinity, aridity causing stress to seeds tending for irregular germination are causes of no seed germination (Heydecker and Coolbear 1977). In this study, standard germination and output performance with early seedling growth of some oil and confectionery sunflower cultivars was investigated.

MATERIALS -METHODS

This study was conducted at the laboratories of Variety Registration and Seed Certification Center, Ankara. For the confectionary cultivar of sunflower Palancı 1, Çiğdem 1 and İnegöl Alası and for the oil sunflower cultivar Tunca, Sanbro and 08 TR 003 were used as materials. During laboratory analyses; germination containers containing silt sand (0,8 mm diameter), calibrated growth rooms, sterilisation equipment, electronic moisture meter, assay balance (with a precision of 0,001) seed storage room, oven and desiccator were used.

Laboratory studies were conducted between 15th of January and 15th of April 2014, standard germination tests, sprouting speed, sprouting vigor, germination speed, germination vigor, length of the seedling, length of the root, aboveground wet and dry weight, underground wet and dry weight were measured and weighed. Field output tests were performed at the pilot fields at Yenikent station of the Variety Registration and Seed Certification Center between the 16th of April and 07th of May 2014.

In accordance with the methods and applications of International Seed Testing Association (ISTA) for each cultivar 100 seeds 4 replications are counted. As germination medium containers with a 25 cm length, w5 cm wide, 15 cm deep were used. With closing the germination containers they are let into the germination cabinets working with an accuracy of ± 1 ° C to a pre-set day temperature of 30 ° C and night temperature of 20 °C. Evaluation of the germination were done in accordance with the criteria established in the seed evaluation manual of ISTA. In the evaluation the % ratios of normal germinations, abnormal germination and dead seeds were determined.

RESULTS

The values of analysis of variance results of determined standard germination for the oil and confectionary sunflower cultivars with germination speed, germination vigor, sprouting speed, sprouting vigor are given in table 1.

Table 1: Analysis of variance for the germination speed, germination vigor, sprouting seed and sprouting vigor values determinate as a result of standard germination result of sunflower cultivars.

Source of variation	Degrees of freedom	Mean Square			
		Germination speed	Germination vigor	Sprouting speed	Sprouting vigor
Overall	23	-	-	-	-
Replication	3	6,44	13,56	49,06	9,94
Cultivar	5	239,47**	80,27**	391,37**	145,77**
Error	15	10,04	7,29	16,26	17,68

** $p<0.01$

Values for germination speed and vigor

As shown in Table 1 germination speed, germination vigor, sprouting speed and sprouting vigor values of different confectionary and oilseed sunflower cultivars were statistically significant at the 1% level in term of cultivars.

When analysing the values of germination speed it is determined that the highest value belongs to the oil sunflower cultivar Tunca with 96,50 % and this is followed with 90 % by oil sunflower cultivar 08 TR 003 and the lowest germination speed value is obtained with 77 % from the confectionary sunflower cultivar Çiğdem 1.

In terms of germination vigor the highest value is determined with 98 % by the cultivar Tunca followed by a value of 96 % by oil sunflower cultivar 08 TR 003 and lowest value of 86 % from the confectionary sunflower cultivar Çiğdem 1. (Table 2)

Table 2: Germination speed and vigor values (%) of sunflower cultivars determined as a result of standard germination

Cultivar	**Germination speed**	**Germination vigor**	**Cultivar**	**Germination speed**	**Germination vigor**
Palancı 1	79,00 c	90,00 cd	**Tunca**	96,50 a	98,00 a
Çiğdem 1	77,00 c	86,00 d	**Sanbro**	87,50 b	95,50 ab
İnegölAlası	79,00 c	91,50 bc	**08 TR 003**	90,00 b	96,00 ab
Germination speed LSD $_{0.01:}$ 4.78			Germination vigor LSD $_{0.01:}$ 3.48		
Germination speed VK : 3.74			Germination vigor VK : 2.91		

Values for sprouting speed and vigor

When analysing the mean values of cultivars at the table 3 it is seen that the highest value belongs in terms of sprouting speed with 95,50 % and the sprouting vigor with 95,60 % from the oil type sunflower cultivar Tunca.

Table 3. Sprouting speed and vigor values (%) of sunflower cultivars determined as a result of standard germination

Cultivar	**Sprouting speed**	**Sprouting vigor**	**Cultivar**	**Sprouting speed**	**Sprouting vigor**
Palancı 1	72,50 c	86,50 b	**Tunca**	95,50 a	96,50 a
Çiğdem 1	71.50 c	78,50 c	**Sanbro**	82,50 b	84,50 bc
İnegölAlası	76,00 c	86,00 b	**08 TR 003**	90,50 a	90,50 ab
Sprouting speed LSD $_{0.01:}$ 6.08			Sprouting vigor LSD $_{0.01:}$ 6.34		
Sprouting speed VK : 4.95			Sprouting vigor VK : 4.83		

Seedling length

When analysing the values for seedling length, it is determined that the highest value of 19,47 cm seedling length of oil type cultivar Sanbro followed with a value of 18,64 cm by the oil sunflower cultivar Tunca and the lowest seedling length with 16,48 cm of confectionary sunflower cultivar Palancı 1.

Root length

When analysing the mean root length values the highest root length was obtained with 11,55 cm from the oil sunflower cultivar Tunca.

Above – ground wet weight

In terms of mean above ground wet weight the highest value was determined with 7,70 g. by oil sunflower cultivar 08 TR 003

Above – ground dry weight

In terms of mean above ground dry weight it is determined, that the highest value belongs with 0,60 g to the confectionary sunflower cultivar İnegölAlası followed with 0,56 g by confectionary sunflower cultivar Palancı 1 and the lowest value obtained with 0,33 g from the oil sunflower cultivar Tunca.

Below – ground wet weight

In terms of mean below ground wet weight it is determined, that the highest value belongs with 1,80 g to the confectionary sunflower cultivar İnegöl Alası followed with 1,73 g by confectionary sunflower cultivar Palancı 1 and the lowest value for below ground wet weight is obtained with 1,12 g from the oil sunflower cultivar Sanbro.

Below – ground dry weight

The highest below ground dry weight is obtained with 0,25 g by confectionary sunflower cultivar and the lowest below ground dry weight with 0,07 g from oil sunflower cultivar 08 TR 003.

Field output vigor

In terms of field output vigor values for the cultivars the highest field output belongs with 91,50 % to the oil sunflower cultivar Tunca followed with 86 % by oil sunflower cultivar 08 TR 003 and the lowest germination vigor value obtained with 75 % from the confectionary sunflower cultivar Çiğdem 1.

CONCLUSION

Evaluating the data obtained from the study in optimal and controlled manner, it was possible to distinguish genetic structure after measurements of seedling length, root length, above ground wet and dry weights, below ground wet and dry weights. Besides laboratory based data the data was also taken out of field conditions that also affirmed similar trends despite some minor differences similar. In addition to the genetic structure environmental factors were also effective under field conditions.

When comparing the germination speed and vigor values of oil and confectionary cultivars it is observed that germination vigor of oil cultivars are higher than others.

In terms of field output vigor it is determined that there are no important differences between oil and confectionary cultivars. It is to be pointed out, that the oil sunflower cultivar Tunca is the best among cultivars tested under field conditions.

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DETERMINATION OF ACCELARATED AGING AND FIELD GERMINATION TEST VALUES OF SOME CONFECTIONARY AND OILSEED SUNFLOWER (*HELIANTHUS ANNUUS* L.) CULTIVARS

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ABSTRACT

It is very important to know sowing performance for production of sunflower that has wide economic importance. It is very common to observe variation in performance of same plant, the same cultivar seeds, even if the they have same chronological age class during their field propagation and performance. Performance difference among seed lots is due to variation in seed vigor. This study was carried out in the laboratory of the "Variety Registration and Seed Certification Center" between 15 January – 15 April 2014 to determine the germination and seedling growth of some confectionary and oilseed sunflower cultivars namely Palancı 1, Çiğdem 1 and İnegöl Alası and Tunca, Sanbro and 08 TR 003 respectively. In the study, standard germination tests measuring germination speed and germination vigor, seedling length, root length, above-ground fresh and dry weight, below-ground wet and dry weight values. According to the germination speed and germination vigor values, the oilseed cv. Tunca of sunflower that had the highest values of 96,50% and 98 %, and confectionary type cv. Çiğdem1 had the lowest value of 77 % for germination speed and lowest value of 86% in germination vigor in terms of the general average for all cultivars.

Key Words: Sunflower (*Helianthus annuus* L.), Accelerated Aging, Field Germination Vigor

INTRODUCTION

Oilseed plants within vegetative production are defined as vital basic necessities for human nutrition (Yurdagül and Ersoy, 1997). With regard to cultivation area and production of oilseed plants sunflower has first place containing high proportion of oil (22-50 %) it is an important vegetable oil plant for raw oil production. (Kızıloğlu, 1992., Kara,1996). We meet 46 % of our oil demands from sunflower(Anonymous, 2007). Furthermore 2,6 % of sunflower is consumed for confectionery purposes (Arıoğlu, 2000). Sunflower seed which is also rich in terms of potassium and vitamin E is an important source of linoleic acid. Foods which are rich in terms of linoleic acid are helping to decrease the cholesterol level in blood (Anonymous, 2004).

It is very important to know the planting values at the production phase of sunflower that has a wide area of usage and economic importance. As the seeds growing on sunflower table mature at different times, therefore, sunflower seeds performance varies even we take same kinds of seeds from the same plants under field conditions and in their germination performances. These performance differences observed between seed samples arise from seed vigor (ISTA 1995).

In Turkey, there is no legal obligation for performing vigor test on other cultivars as well as on sunflower seeds parties. Therefore standard germination test performed by seed producer companies are considered as adequate and performing of an additional vigor test is considered unnecessary. A vigor test allows us to obtain reliable opinion on estimating the quality of seed samples, classification, output and their storage capabilities.

Vigor test is a test performed to measure the performance and vigor out of the vitality value of seed sample. To determine the seed vigor, various tests such as electrical conductivity test (EC), controlled deterioration test, cold test (CT), cool germination test (CG) and accelerated aging tests (HY) (Anonymous, 2013).

The aim of seed vigor test is; to be aware of sowing values for a wide range of environments and estimating the outputs in unfavourable planting conditions besides their classification of seed samples and also to obtain data for storing potentials of seeds. Seed vigor in a broad sense represents the sum of the properties confirming the activity and performance of seeds.

At the discussions of seed qualities a quality variable on agenda in recent years is a feature of seed vigor. The seed vigor feature as a basic quality feature of seed can be defined as a measure of germination and formation of plantlets potential get through all the hindering stress factors under uncontrolled field conditions.

ISTA describes the seed vigor as a sum of features that performing germination activities and performances under various environmental conditions of seeds. And also with the feature to give an opinion for formatting a plant under field condition of seed, given a chance for seed inventory management demonstrates the importance of the possibility of seed vigor measurement (Başak, 2006).

MATERIALS and METHODS

This study was conducted at the laboratories of Variety Registration and Seed Certification Center, Ankara as accelerated aging studies on confectionary and oilseed sunflower cultivars between the dates of 15th of January and 15th of April 2014. All materials used in the study were obtained from the harvest of the year 2013. As cultivars of confectionary sunflower Palancı 1, Çiğdem 1 and İnegöl Alası and as oil sunflower the cultivars Tunca, Sanbro and 08 TR 003 were used as materials.

For laboratory analysis; at the accelerated aging tests aging containers consisting of three parts were used. Aging container consisted of an outer box with a size of 11×11×4 cm and inside thereof from sieve mesh made inner chamber with a size of 10×10×3 cm and a cover on the container (Figure 1). With the aim to maintain a high relative humidity 40 ml pure water was put in the outer chamber. Seeds are spread on sieve mesh in a single layer and the sieve mesh was placed into the outer container. At this stage, it is important not to splash water on sieve mesh and seeds. After seeds are put on the mesh the cover was closed. At all seed samples from each 100 seeds 4 replications were applied. In addition to this also necessary laboratory equipment was used. Thousand grain weight and grain moistures of cultivars was determined before the start of the test.

Accelerated aging containers were left in the aging chamber operated at 41 ºC. Aging was performed for duration of 24-48-72 and 96 hours. Temperature control of aging chamber was made on a continuous and regular basis. 1 hour after getting the seeds out of the aging chamber a standard germination test in accordance to the rules of ISTA was applied with seeds in each of 4 replications.

Statistical analysis and evaluation**:** Intra-period comparison values obtained in the study were subjected to analysis of variance according to the factorial randomised randomized blocks design",

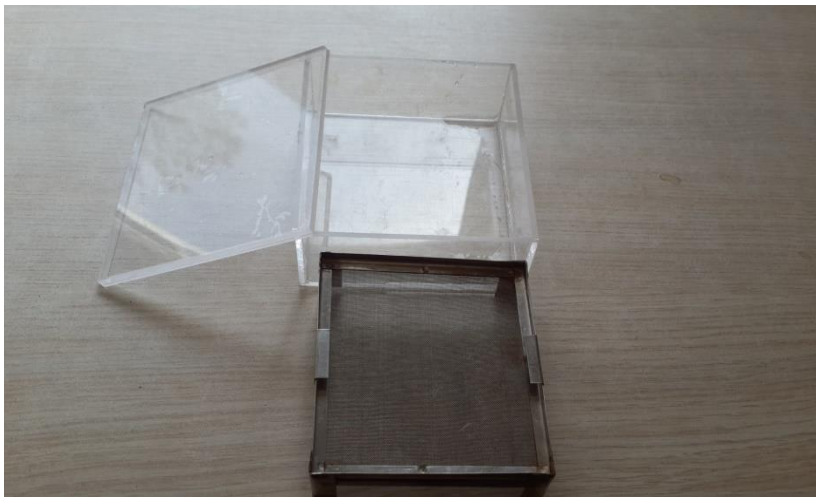

Figure 1. Accelarated aging Equipments

RESULTS AND DISCUSSION

Germination vigor values after 24 hours of accelerated aging

Analysis of variance results for the germination vigor test results of accelerated aging for a duration of 24,48,72 and 96 hours for oil and confectionary type sunflower cultivars are given in table 1.

Table 1: Analysis of variance for the germination vigor values determined after an accelerated aging test of 24, 48, 72 and 96 hours applied to sunflower cultivars.

Source of variation	Degrees of freedom	Mean Square			
		24 hours	48 hours	72 hours	96 hours
Total	23	------	-----	-----	-----
Replication	3	5.83	40.50	63.83	1.26
Cultivar	5	174.07**	596.97**	852.58**	821.14**
Error	15	5.33	10.50	3.88	15.70

** $P<0.01$

As shown in Table 1 germination vigor values after 24, 48, 72 and 96 hours of accelerated aging of different confectionary and oilseed sunflower cultivars were statistically significant at the 1% level on the basis of cultivars.

When analysing average germination vigor values of accelerated aging for 24 hours of sunflower cultivars it is seen that the highest value with 94,50 % belongs to the oil sunflower cultivar Tunca and this is flowed with 91,75 % by oil sunflower cultivar Sanbro and the lowest germination vigor value is getting by confectionary sunflower cultivar Çiğdem 1 with a value of 75.50 % (Figure 2).

When analysing average germination vigor values from the table 3 of accelerated aging for 48 hours of cultivars it is seen that the highest value with 88,50 % belongs to the oil sunflower cultivar Tunca and this is followed with 73,25 % by oil sunflower cultivar 08 TR 003 and the lowest germination vigor value of 50,25 % was noted on confectionary sunflower cultivar İnegöl Alası.

When analysing average germination vigor values from the table 4 of accelerated aging for 72 hours of cultivars it is seen that the highest value with 82,50 % belongs to the oil sunflower cultivar Tunca and

this is followed by 55,75 % by confectionary sunflower cultivar Palancı 1 and the lowest germination vigor value was noted as with 37,45 % by confectionary sunflower cultivar İnegöl Alası.

Table2: Germination vigor (%)values determined after 24 hours of accelerated aging test by cultivars

Cultivar	**Accelerated Aging (HY) -24 hours**	**Cultivar**	**HY-24 hours**
Palancı 1	87,75 c	**Tunca**	94,50 a
Çiğdem 1	75,50 d	**Sanbro**	91,75 ab
İnegöl Alası	90,00 bc	**08 TR 003**	89,00 bc
LSD $_{0.01:}$ 3.48 VK : 2.62			

Table 3: Germination vigor (%)values determined after 48 hours of accelerated aging test by cultivars

Cultivar	**HY-48 hours**	**Cultivar**	**HY-48 hours**
Palancı 1	71,25 b	**Tunca**	88,50 a
Çiğdem 1	68,75 b	**Sanbro**	71,50 b
İnegöl Alası	50,25 c	**08 TR 003**	73,25 b
LSD $_{0.01:}$4.88 VK : 4.60			

Table 4: Germination vigor (%)values determined after 72 hours of accelerated aging test by cultivars

Cultivar	**HY-72hours**	**Cultivar**	**HY-72hours**
Palancı 1	55,75 b	**Tunca**	82,50 a
Çiğdem 1	47,00 d	**Sanbro**	51,00 c
İnegöl Alası	37,45 e	**08 TR 003**	52,00 c
LSD $_{0.01:}$2.99			
VK : 3.60			

When analysing average germination vigor values from the table 4.27 of accelerated aging for 96 hours of cultivars it is seen that the highest value with 60,25 % belongs to the oil sunflower cultivar Tunca and this is followed with 37,75 % by oil sunflower cultivar Sanbro and the lowest germination vigor value of 21.75 % was obtained on confectionary type sunflower cultivar İnegöl Alası.

Table 4. Germination vigor (%)values determined after 96 hours of accelerated aging test by cultivars

Cultivar	**HY-96Hours**	**Cultivar**	**HY-96hours**
Palancı 1	24,00 c	**Tunca**	60,25 a
Çiğdem 1	25,50 c	**Sanbro**	37,75 b
İnegöl Alası	21,75 c	**08 TR 003**	35,00 b
LSD $_{0.01:}$5.97			
VK : 11.64			

CONCLUSION

As a result of variance analyses performed in terms of accelerated aging test values between six different cultivars a statistic difference of 1 % is determined. When analysing aging values of cultivars with a duration of 24, 48, 72 and 96 hours it is determined that the highest value with 94,50 % belonged to the oil sunflower cultivar Tunca and the lowest germination vigor value 75,50 % belonged to the confectionary sunflower cultivar Çiğdem 1. As the result of accelerated aging for 48 hours it is determined that the highest value with 88,50 % belonged to the oil sunflower cultivar Tunca and the lowest germination vigor value belonged to 50,25 % for confectionary sunflower cultivar İnegöl Alası. As the result of accelerated aging for 72 hours it is determined that the highest value with 82,50 % belonged to the oil sunflower cultivar Tunca and the lowest germination vigor value of 37,45 % belonged to confectionary type sunflower cultivar İnegöl Alası. As a result of accelerated aging for 96 hours it was determined that the highest value with 60,25 % belonged to the oil sunflower cultivar Tunca and the lowest germination vigor value of 21,75 % belonged to the confectionary sunflower cultivar İnegöl Alası.

When comparing the accelerated aging values for 24, 48, 72 and 96 hours for oil and confectionary cultivars it was observed that the accelerated aging values of oil cultivars are higher compared to confectionary type cultivars.

A steady decline in the germination rate of 24-48-72 and 96 hours aging process was observed for sunflower cultivars. Critical point for the sunflower cultivars used in this study is the accelerated aging test performed for 96 hours. It is to mention, that each aging performed over 96 hours can reduce the germination vigor and perhaps be a dead end for some cultivars (İnegöl Alası and Çiğdem 1). Accordingly if an accelerated aging test has to be performed for sunflower, it is recommended to carry out for 96 hours.

As a result of the study; when the result get from the accelerated aging increase an increase at the germination vigor, sprouting vigor and field output vigor were observed. We can indicate for the cultivars having a great acceleration aging result, that this cultivar is resistant for storage, transport and also has a higher field output rate. To determine the storage, transport and field output values is not a determining factor for germination vigor and sprouting vigor. Since germination and sprouting vigor tests are conducted under controlled conditions and without forcing the seeds. But at the accelerated aging test the seed is aging with fatiguing through moisture and temperature and germination value is determined after this aging.

As a result observed by the cultivars of sunflowers used the accelerated aging values are higher by oil cultivars compared to confectionary cultivars. It is to point out, cultivar Tunca is a superior cultivar among all cultivars in terms of transport, storing and field output.

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