Application of Method Embryo Culture in Combination with Gamma Irradiation and Ultra Sonic (Part II)

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Abstract

Lines resistant to *Plasmopara halstedii*, *Phomopsis helinthi*, *Septoria helianthi* and *Orobanche cumana* (race E and G) were obtained from the Bulgarian susceptible fertility restorer lines 2574 R, 147 R, 374 R, 381 R, 377 R, American line RHA-857 and Bulgarian line with normal cytoplasm 197 B. Lines were developed using embryo culture method in combination with ultra sonic and gamma irradiation at sunflower immature zygotic embryos. All investigated lines possessed 100% resistance to diseases and parasite *Orobanche*. Some of the new lines showed very good combining ability. Line 12002 R developed through embryo culture method at immature zygotic embryos in combination with gamma irradiation at dose 8 Gy is paternal component of commercial hybrid Rada. Line 12003 R developed through embryo culture method at immature zygotic embryos in combination with ultra sonic at dose 25.5 W cm^{-2} for 1 min is paternal component of commercial hybrid Yana. Hybrids Rada and Yana possessing immunity to parasite *Orobanche cumana*race E and G, respectively; immunity to *Plasmopara helianhi* – races 300, 330, 700 and 731; resistance to *Phomopsis*; immunity to *Macrophomin*a and tolerance to *Phoma*. Our results showed that mutagenesis in sunflower can be successfully applied to develop new lines suitable for use as parental components of new hybrids.

Keywords: embryo culture method; gamma rays; Helianthus annuus L.; Macrophomina phaseolina; new lines; parasite Orobanche cumana; Phoma macdonaldii; Phomopsis helinthi; Plasmopara halstedii; resistance; Septoria helinthi;sunflower; ultra sonic

Introduction

Through the years, mutagenesis has generated a vast amount of genetic variability and has played a significant role in plant breeding programs throughout the world. Records maintained by the joint FAO/IAEA Division in Vienna show that 2,965 crop cultivars, with one or more useful traits obtained from induced mutations, were released worldwide during the last 40 years (FAO-IAEA, 2011). Using radiation breeding, traits for yield, quality, taste and disease and pest resistance have been improved in cereals, legumes, cotton, peppermint, sunflower, peanut, grapefruit, sesame, banana and cassava. Basic scientific research has substantially benefited from mutagenesis. Mutation breeding has substantially contributed to the countries' economies and to the conservation of biodiversity by stopping genetic erosion. Improvement of crop production regarding disease and pest management is one of the main goals in agriculture. Pathogens cause huge yield losses in the agriculture every year with large economic losses and damage to ecosystems.

Induced mutations will continue to have an increasing role in creating crop varieties with traits such as modified oil, protein and starch quality, enhanced uptake of specific metals, deeper rooting system and resistance to drought, diseases and salinity as a major component of the environmentally sustainable agriculture (Ahloowalia *et al*., 2004). The author summarized that the economic value of a new variety can be reduced use of pesticides and fungicides (e.g. in varieties resistant to diseases and insect pests).

Mutagenesis, both physical and chemical, proved favourable for mutation induction in tissue cultures. It is a technique which allows widening a heritable variability by inducing new traits. Some of them can be of interest as agronomical important characters; others can be used as marker traits. Induced mutagenesis by chemical or radiation mutagens has advantages over insertion methods, since mutagens introduce random changes throughout genome and can generate variety of mutations within a single plant (Kozjak and Meglič, 2012).

Mutant forms resistant to *Orobanche cumana* were produced after treatment of ear dry seeds of open-pollinated varieties VNIIMK and Start with gamma rays (Christov *et al*., 1998). Mutation for resistance to *Verticillium* was obtained when induced mutagenesis and tissue cultivation were combined

appropriately in tomato (Gavazzi *et al*., 1987). Kostov *et al*. (2007) increased resistance of tomato to *Orobanche ramosa* L. by application of Ethyl metansulfonate (EMS).

Sunflower is one of the most important oil crops in the world. The development of variable breeding material is a primary task of the genetic and breeding programs in this crop. Although sunflower breeding has been very successful throughout the last decades, a number of aims remain to be achieved, e.g. resistance to various diseases and the parasite *Orobanche*. However, these efforts are obviously limited by the narrow genetic base of commercial sunflower which has to be enlarged by the utilization of new approach as tissue culture in combination with induced mutagenesis. Therefore, development of new lines resistant to diseases and parasite broomrape is very important for sunflower breeding.

Plasmopara halstedii has been observed in western European sunflower crops since 1960 (Leppik, 1962). The disease is caused by the Oomycete *Plasmopara halstedii* (Farl.) Berl. et de Toni, specific to sunflower and causing infection from soil or from seed. Different physiological races (pathotypes) can attack a variable range of sunflower genotypes (Tourvieille de Labrouhe, 1999). Downy mildew is a major disease in the regions with mass production of sunflower. The incidence of downy mildewed sunflowers in a field may range from traces to near 50% or even up to 95% (Sackston, 1981).

Grey spots caused by *Phomopsis helianthi* and black spot (*Phoma macdonaldi*) are serious problem for sunflower production in a number of countries worldwide (Carre, 1993; Škorić, 1994; Gulya, 1997), including Bulgaria (Encheva and Shindrova, 2011). The disease has epiphytotic outbreaks in years with hot and moist weather during sunflower vegetation.

Septoria leaf blight of sunflower, caused by *Septoria helianthi* Ellis and Kellerman is widely distributed throughout the world. The disease is a major contributor to sunflower yield losses and is most destructive under conditions of abundant rainfall (Saharan and Singh, 1976; Kubenkova, 1980; Liu and Lu, 1988; Yang *et al*., 1988).

Macrophomina phaseolina causal agent of charcoal rot is a serious threat for sunflower crop especially in the arid regions of the world (Hoes, 1985). It is responsible for causing losses on more than 500 cultivated and wild plant species (Indera *et al*., 1986). The fungus is reported to be soil, seed and stubble borne. The infected plants show early maturity, reduced head size and fewer numbers of grains setting (Meyer *et al*., 1974). Yield losses claimed by charcoal rot in Spain, United States, Uruguay and Soviet Union up to 25% have been recorded; however under favourable conditions for

the growth and development of the *M. phaseolina*, total failure of the crop in specific areas has been recorded (Tikhonov *et al*., 1976; Jimenez *et al*., 1983).

Broomrape (*Orobanche cumana* Wallr.) is a holoparasitic plant that causes serious damages to sunflower production, especially in Central and Eastern Europe, Spain, Turkey, Israel, Iran, Kazakhstan and China (Škorić *et al*., 2010). According to Kaya *et al*. (2004), about 80% of the sunflower areas in Turkey (Trakia region) are infested with seeds of the parasite. According to these authors, every 20 years there is epiphytotic occurrence of broomrape in the region. Furthermore, the parasite forms new more virulent races which overcome the resistance of the varieties and hybrids commonly used in production (Alonso, 1996; Pacureanu-Joita *et al*., 1998; Fernandez-Martinez *et al*., 2008; Kaya *et al*., 2004). This complicates the control of broomrape.

Broomrape presents serious problems to sunflower production in Bulgaria, as well. It occupied area of 690,000 ha. This has caused a systematic disturbance of proper crop rotation. Sunflower is grown on the same field in every second or third year. It is constantly expanding its distribution area, forming new more virulent races. Race F was identified after 1995, race E after 2003 and race G after 2005 (Shindrova and Penchev, 2012). The population identified as race H was isolated in 2007, only on a very small area. Although a large number of *Orobanche*-resistant hybrids have been developed during the last years, there still are large areas infected with this parasite (Shindrova and Penchev, 2012). This leads to considerable losses expressed, on the one hand, in yield decrease, and, on the other hand, in worsened quality of the obtained production (Shindrova *et al*., 1998).

Besides chemicals and good agronomy practices recommended for control of diseases and broomrape on sunflower, the breeding method is the most efficient and economically most advantageous. The developing of resistant lines, hybrids and their introduction in practice will lead to restrict propagation and distribution of the pathogens, and in the course of time – cleaning of the infected fields (Škorić, 1985, Vear *et al*., 1997, Hahn and Degener 1999, Roustaee *et al*., 2000, Shindrova and Penchev, 2012).

The aim of this study was:

- (a) to summarize mutations for resistance to *Phomopsis helinthi*, *Plasmopara halstedii*, *Septoria helianthi* and parasite *Orobanche cumana* at sunflower R and B lines, developed through

embryo culture method in combination with ultra sonic and gamma irradiation at different initial susceptible genotypes and

- (b)

to characterize new hybrids Rada and Yana, developed with mutant lines according to their resistance to diseases and parasite broomrape.

Materials and methods

A part of the experiments were carried out under laboratory conditions, and another – at the trial field of Dobroudja Agricultural Institute – General Toshevo.

Developing of mutant lines

The Bulgarian fertility restorer lines 2574 R, 147 R, 374 R, 381 R, 377 R, American line RHA-857 and Bulgarian line with normal cytoplasm 197 B, which is highly homozygotic, were used as donor materials. A main requirement to the initial plant material used according to the methods of embryo culture in combination with ultra sonic or gamma irradiation is to be genetically pure, i.e. homozygotic to the highest possible degree. Therefore, the donor lines with very good morphological uniformity were chosen as initial material for induced mutagenesis.

Plants were grown in the field and were hand-pollinated. The immature seeds (13–16 days old) were treated with ultrasound at dose 25.5 W cm^{-2} for 1, 2, 3, 5, 7, 9, 10, 11, 13 and 30 min and ionizing radiation such as gamma rays (^{137}Cs) at dose 8 Gy and 50 Gy (the power of the dose being 0.338 krad min^{-1} = 3.38 Gy min^{-1}). The immature seeds were sterilized under the following conditions:

- (1)

1 min in 95% ethanol;

- (2)

15 min in bleaching solution (2.7% Cl);

- (3)

followed by several washings with sterile distilled water.

The immature zygotic embryos were aseptically isolated and planting on nutrition medium M for further growing (Azpiroz *et al*., 1988): 1/2 MS (Murashige and Skoog, 1962) macro salts, MS micro salts, B5 vitamins (Gamborg *et al*., 1968), 20 g l^{-1} sucrose, pH – 5.7. The conditions for cultivation were: 25°C, 16/8 h photoperiod for 1 week. R0M0 plants (nomenclature according to Novak *et al*., 1988), which

formed roots, were transplanted for further grown, self-pollinated and harvested by single plants under greenhouse conditions. The seeds produced (R1M1) were sown in the field.

Phytopathological evaluation

The phytopathological evaluation of the Bulgarian fertility restorer lines 2574 R, 147 R, 374 R, 381 R, 377 R, American line RHA-857, Bulgarian line with normal cytoplasm 197 B, mutant R and B lines and hybrids Rada and Yana were performed with regard to *Orobanche cumana* and *Plasmopara halstedii* at the Sunflower Phytopathology Laboratory; to *Phomopsis helianthi* and *Phoma macdonaldii* at infection fields of DAI – General Toshevo; to*Septoria helianthi* and *Macrophomina phaseolina* at natural conditions in field of DAI – General Toshevo.

The phytopathological evaluation of lines and hybrids was performed with regard to *Downy mildew* (*Plasmopara halstedii* (Farl.) Berlese & de Toni) – race 300, 330, 700 and 731. The method suggesting by Gulya *et al*. (1991)was used with a view to characterizing the resistance to *Plasmopara halstedii*. The evaluation of 50 plants from line was carried out using standard methodology:

- 0% = S (sensitive);
- 100% = R (resistant).

Broomrape resistance (race E and G) was evaluated under greenhouse conditions according to Panchenko (1975), slightly modified to the local conditions. A mixture of soil and sand (2:1) was prepared, and 0.2 mg of broomrape seeds was added to each kilogram of the mixture. Sunflower was grown in this substrate in the following order: 50 plants + 10 plants (standard-AD-66) in every container. They were placed in a greenhouse under controlled conditions with irrigation. Forty-five days after planting, the roots of all sunflower plants were cleaned and checked for the existence of the parasite. Broomrape resistance was calculated as percentage of non-infected plants. The reaction of 50 plants from each line was recorded using the following scale:

- 0% = S (sensitive);
- 100% = R (resistant).

The evaluation for resistance to attacks of grey spots (*Phomopsis helianthi*) and black spots (*Phoma macdonaldii*) was performed in filed and in artificial infection plots. The type and severity of the attack were read 1 week after mass flowering according to the following scales:

Type of grey spot attack:

- 0–no symptoms;
- 1–a necrotic lesion up to 5 cm in diameter;
- 2–a necrotic lesion over 5 cm in diameter,
- 3–several merged necrotic lesions on the stem,
- 4–a stem broken at the place of infection.

Type of black spot attack:

- 0–no symptoms;
- 1–a necrotic lesion near the petiole;
- 2–several necrotic lesions on the stem;
- 3–the entire stem is covered with necrotic lesion or the stem is broken.

Attacking rate: what portion of the plant's stem is covered with lesions of the pathogen (1/3, 2/3 or 3/3) (Encheva and Kiryakov, 2000).

The evaluation for resistance to attacks of septoria (*Septoria helianthi* Ellis and Kellerman) was made in August at natural conditions in field when seed fill was in progress, and disease development was near its peak. The evaluation of 50 plants from line was carried out using a 1–4 scale, with values of 1 and 2 corresponding to highly and moderately resistant plants, respectively, and 3 and 4 corresponding to moderately and highly susceptible plants, respectively.

Macrophomina phaseolina resistance was evaluated at natural conditions in field of Dobroudja Agricultural Institute – General Toshevo. The evaluation of 50 plants from line was carried out using standard methodology:

- 0% = S (sensitive);
- 100% = R (resistant).

Results and discussion

Immature sunflower (*Helianthus annuus* L.) zygotic embryos of sunflower R and B lines were treated with ultra sonic and gamma rays before plating to embryo culture medium. *In vitro* mutagenesis was wedding with embryo culture method, which allowed isolation of embryos before terminating their development and their plating in nutrition medium to grow *in vitro* seedlings. New lines were developed at sunflower immature zygotic embryos.

Evaluation of R and B lines for resistance to some economically important diseases and parasite on sunflower

In Bulgaria where sunflower is grown commercially, the successful production is endangered by many fungal pathogens and parasites. Losses may be severe, near 100% in parts or even entire fields under extreme circumstances. Although sunflower breeding has been very successful throughout the last decades, a number of aims remain to be achieved, e.g. resistance to various diseases and the parasite *Orobanche*. However, these efforts are obviously limited by the narrow genetic base of

commercial sunflower which has to be enlarged by the utilization of few techniques as induced mutagenesis in particular. It is an alternative method to conventional ones.

Tab.
*1
Table 1:

Sunflower lines resistant to *Plasmopara halstedii*, *Phomopsis helinthi*, *Septoria helinthi* and parasite *Orobanche cumana*, developed through embryo culture method in combination with ultra sonic and gamma irradiation

In our study, plants resistance to various diseases and parasite *Orobanche cumana* were observed (Table 1). The initial R and B genotypes were susceptible. These results were confirmed during three years of evaluation.

Figure 1:

Phomopsis helianthi

Phomopsis helianthi has epiphytotic outbreaks in years with hot and moist weather during sunflower vegetation. Pathogen overwinters in plant residues in the form of peritecii. Infection became in phase 6–8 pair of leaves with ascospores of the fungus. According to Škorić (1985), *Phomopsis* is the most

destructive pathogen of sunflower. In extreme cases, it can compromise sunflower production and cause significant yield losses.

Resistance to *Phomopsis helinthi* was registered at line 106 R, received after treatment of susceptible American line RHA-857 (Figure 1) with ultra sonic at dose 25.5 W cm^{-2} for 3 min (Encheva, 2009b). Three years of phytopathological evaluation of the new line showed stable inheritance in the progenies.

Plasmopara halstedii is a soil-borne pathogen, its oospores serving as primary inoculums for young sunflower seedlings. The oospores (resting spores) capable of surviving for as long as 8–10 years in the soil. Till 2000 there are 10 downy mildew races existing in the world, as follows: 100, 300, 310, 330, 700, 703, 710, 711, 730 and 770 (Tourvieille de Labrouhe *et al*., 2000). According to Khan (2007), new races appear frequently owing to the pathogenic variability in the fungus and the selection pressure resulting from the use of resistant sunflower cultivars and fungicide seed treatment. In 2007, Gulya reported 35 races in different parts of the world. Since 2005 it was established race 330 in North-East part of Bulgaria (Shindrova, 2006).

In our work, we succeeded to receive resistant to *Plasmopara halstedii* lines 97 R, 99 R, 100 R and 101 R, after treatment of susceptible Bulgarian line 381 R (Figure 2) with ultra sonic at dose 25.5 W cm^{-2} for 1 min and line 98 R for 3 min (Encheva and Shindrova, 2011).

Figure 2:

Plasmopara halstedii

A number of major resistance genes to downy mildew have been either identified in cultivated sunflower or introduced from wild *Helianthus* species (Miller, 1992). In our experiment, we prove that 100% stable resistance of the sunflower mutant lines to downy mildew (race 330) can also be obtained through induced mutagenesis, by treatment of immature zygotic embryo with ultra sonic, in particular. Resistance to *Plasmopara halstedii*, was observed in several plants, obtained after treatment of Bulgarian genotype 381 R with ultra sonic. This allows us to assume that there are mutable locations in the cultural sunflower genome resulting from induced mutagenesis.

Septoria helianthi winter as stroma and picnidii of plant residues. In mass attack observed tearing lamina and result was leaf drop. Infected plants have low yield, and seeds contain a lower percentage of oil (Yang *et al.*, 1988). Resistance to *Septoria helianthi* was observed at lines 143 R (Figure 3) and 145 R, received after treatment of susceptible Bulgarian line 377 R with ultra sonic at dose 25.5 W cm^{-2} for 1 min and 2 min, respectively (Encheva, 2013). The genetic nature and inheritance of *Septoria* leaf blight resistance is not known (Block, 2005).

Figure 3:

Genotype 377 R (left) susceptible to *Septoria* and line 143 R (right) resistant to *Septoria*

Orobanche cumana (Figure 4) in sunflower is one of the most important problems to solve because it causes drastic decreasing of yield. The parasite *Orobanche cumana* grow on sunflower roots, resulting in weak and dwindled plants, with thin steam. The parasite enhances the transpiration of damaged plants, which in drought conditions are withering, even if attacked by a small number of parasitic plants (Iliescu *et al*., 1998). *Broomrape*presents serious problems to sunflower production in Bulgaria, as well (Shindrova, 1994).

Resistance of the new lines to broomrape races E and G distributed in Bulgaria was established. The Bulgarian fertility restorer lines 2574 R, 147 R, 374 R and Bulgarian line with normal cytoplasm 197 B were susceptible to this parasite. We succeeded to receive new lines resistant to broomrape after treatment of Bulgarian line 2574 R with gamma irradiation at dose 8 Gy (line 114 R) and ultra sonic at dose 25.5 W cm^{-2} for 1 min (lines 115 R and 116 R) (Encheva *et al*., 2003); at lines 116 R, 117 R, 118 R, 119 R and 120 R developed by treating of Bulgarian line 147 R with ultra sonic at dose 25.5 W cm^{-2} for 5, 7, 9, 11 and 13 min, respectively (Encheva *et al*., 2008); at lines 193 R and 194 R produced after treatment of Bulgarian genotype 374 R with gamma ray at dose 50 Gy and ultra sonic at dose

25.5 W cm^{-2} for 10 min, respectively (Encheva, 2009a) and at line 12003 R, developed by treating of Bulgarian line 2574 R with ultra sonic at dose 25.5 W cm^{-2} for 1 min (Encheva *et al*., 2012a).

Figure 4:

Orobanche cumana

Resistance to *Orobanche cumana* was established at new lines with normal cytoplasm 74 B, 78 B, 85 B and 88 B, also. They were received after treatment of susceptible Bulgarian line 197 B with ultra sonic at dose of 25.5 W cm^{-2} for 1, 3 and 5 min (Encheva *et al*., 2010).

Resistance to parasite broomrape was received simultaneously in several sunflower plants (developed in lines) after treatment of immature zygotic embryos of susceptible Bulgarian lines 2574 R, 147 R, 374 R and 197 B using ultra sound or gamma irradiation.

Several plants (developed in lines) received after treatment of susceptible Bulgarian line 381 R with ultra sonic showed simultaneously resistance to *Plasmopara halstedii*.

Resistance to *Septoria helinthi* was observed at two plants (developed in lines), received after treatment of susceptible Bulgarian line 377 R with ultra sonic.

Resaving of more than one resistant plant from one susceptible genotype allows us to assume that there are mutable locations in the cultural sunflower genome resulting from induced mutagenesis.

Burlov and Kostyuk (1976) and Pogorleckii and Gesele (1976) discovered that broomrape resistance was controlled by a dominant gene which was designated as *Or*.

Our results allow us to presume that the resistance of the mutant sunflower lines to *Orobanche cumana* occurred as a result from a single gene dominant mutation. Similar conclusion has been made by Christov and Nikolova (1996), analyzing the type of resistance to broomrape of mutant sunflower forms obtained through irradiation of air dry seeds with gamma rays. The authors found out that it was controlled by a single dominant gene. Resistant mutants to *Verticillium* with dominant genes were obtained after EMS treatment of pollen of tomato (Gavazzi *et al*., 1987).

Many studies show a monogenic control by a single dominant gene over sunflower resistance against race E (Ish-Shalom *et al*., 1993; Sukno *et al*., 1999), although two dominant genes (Dominguez, 1996) and one recessive gene (Ramaiah, 1987). The race F resistant population BR4, derived from wild species, was found to be under the control of a single dominant gene designated Or_6 (Perez-Vich *et al*., 2002). Pacureanu *et al*. (2004) reported a single dominant gene controlling the resistance to race F in Romania, also. More recent studies on the inheritance to resistance to the latest races in several countries have concluded the presence of one or two dominant genes (Škorić *et al*., 2010; Velasco *et al*., 2012).

Our results confirmed the conclusion of Skirvin (1978), that mutagenesis, physical or chemical, is favourable for induction of mutations in tissue cultures. It was established that the possibilities of experimental mutagenesis in using embryos at an early stage of their development are greater, as compared to air dry seeds (Atanassov, 1988).

Hybrids Rada and Yana, developed with mutant lines 12003 R and 12003 R

Evaluation for resistance to some economically important diseases and parasite on sunflower:

After long inbreeding mutant lines 12002 R and 12003 R were developed and tested for their combining ability. The results from the 3-year testing of lines 12002 R and 12003 R revealed very

good combining ability in hybridization. The line 2607 A (sterile analogues of the Bulgarian inbred line) was used as a tester.

Commercial hybrid Rada is simple cross hybrid, developed by crossing of 2607 *A* × 12002 R. Line 12002 R was developed by embryo culture method of immature zygotic embryos in combination with gamma irradiation at doze 8 Gy (Encheva *et al*., 2003). Hybrid possessing immunity to the parasite *Orobanche cumana* population of race G (resistance of hybrid to race G was established later after screening test): immunity to *Plasmopara helianhi* – races 300, 330, 700 and 731, immunity to *Macrophomina phaseolina* and tolerance to *Phomopsis* and *Phoma*. The immunity to *Orobanche* was inherited from the mutant restorer line 12002 R. Mother line 2607 A, component of hybrids Rada, was susceptible to *Orobanche cumana*.

Commercial hybrid Yana is simple cross hybrid, developed by crossing of 2607 *A* × 12003 R (Encheva *et al*., 2012b). Line 12003 R was developed by embryo culture method of immature zygotic embryos in combination with ultra sonic at dose 25.5 W cm^{-2} for 1 min. Hybrid possessing immunity to the parasite *Orobanche cumana*population of race G, immunity to *Plasmopara helianhi* – races 300, 330, 700 and 731, immunity to *Macrophomina phaseolina*, resistance to *Phomopsis* and tolerance to *Phoma*. The immunity to *Orobanche* was inherited from the mutant restorer line 12003 R, because mother line in the cross-2607 A was susceptible to broomrape.

Resistance of hybrids Rada and Yana to some economically important diseases and parasite *Orobanche* are a guarantee that it is suitable for mass production.

Conclusions

Induced mutagenesis of immature zygotic sunflower embryo allows to develop economically useful traits, including resistance to diseases and to the parasite *Orobanche*.

We succeed to create new sunflower lines resistant to *Plasmopara halstedii*, *Phomopsis helinthi*, *Septoria helianthi*and parasite *Orobance cumana*. According to Sikora *et al*. (2011), the introduction of new genetic variation in inbred elite cultivars offers a unique possibility to identify novel traits, while retaining the agricultural excellence of the lines.

Combining induced mutagenesis in immature zygotic embryo with the embryo culture method, we can conclude that mutation for resistance is only due to the effect of the mutagen. The embryo culture

method alone does not generate variation due to the lack of mutagen factors in the nutrition medium and the short period of *in vitro* cultivation of the immature zygotic embryos.

We concluded that similar changes occurred in several immature embryos of the same genotype. This allows us to assume that there are mutable locations in the cultural sunflower genome resulting from induced mutagenesis. Genetically this regularity could be explained with existence of similarity of quantity of mutable genes in close relative organism.

The available literature on sunflower does not provide data on treatment of immature sunflower zygotic embryos with ultra sonic. In this respect, the approach is especially valuable due to the fact that immature sunflower zygotic embryos are treated at an early stage of development, in which sells were at different face of mitotic cycle; i.e. this is functional tissue. The most sensitive is interphase during which synthesis of DNA is realized (“S” stage). In this phase of mitosis, influence of mutagen factors is strongest and can arouse alterations at nucleus inherited apparatus. This is expected to increase to a higher rate the frequency of mutations in comparison to the classical approach of treating air dry seeds.

The goal in mutagenesis breeding is to cause maximal genomic variation with a minimum decrease in viability. Our conclusion is that ultra sonic causes mutations similar to that of gamma irradiation. Some authors found similar effect of ultra sonic to that of UV, alpha, beta and gamma rays (Beier and Dorner, 1954). In our study, the number of survived plants produced after treatment with ultra sonic was more than that induced by gamma irradiation. This confirms the conclusion of Leung *et al*. (2001) that among the radiation-based methods, gamma radiation causes damage on a larger scale and severely reduces viability.

Mutant lines 12002 R and 12003 R characterized with mutation for resistance to *Orobanche cumana* race E and G were included in heterosis selection. This resistance was successfully transferred to the commercial hybrids Rada and Yana, produced.

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