Genetic variability and identification of markers associated with germination parameters in gamma-irradiation induced mutants of sunflower under water stress condition

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Abstract
The objectives of the present research were to evaluate the variability induced by gamma-irradiation among a population of M8 sunflower mutant lines and to identify molecular markers associated with different seed germination traits. Experiments were carried out under well watered and water-stressed conditions using a randomized blocks design, with three replications. The studied traits consisted of critical times (the time to starting germination, TSG), the time to 50% germination (T50%G) and the time to maximum germination (TMG) and the percentage of seed germination (PSG). In both conditions, a large genetic variation was observed among mutant lines. Some mutant lines showed higher values of germination parameters when compared with the original lines (AS613). Results revealed the a efficiency of gamma irradiation for inducing genetic variation in sunflower, for germination traits. A highly negative, significant correlation was observed between PSG and the critical times of germination. Mutant line "M6-39-2-1" was significantly superior to the original line, for the studied traits, under the water-stressed condition. Multiple regression analyses showed that some AFLP markers were associated with several traits. The most informative AFLP markers were "E37M62-5" and "E33M60-6" which did not have any interactions with water stress. Several markers were associated with seed germination parameters in both conditions. The markers which were associated with different traits could be used in marker-assisted selection for germination parameters.

***Keywords*:** AFLP markers, Mutant, Sunflower, Water stress, Critical times of germination.

INTRODUCTION

Seed germination and early seedling growth are the most sensitive stages to environmental stresses (Cook, 1979; Jones, 1986). Drought stress is one of the most severe limitations of seed germination and crop growth in semi-arid and arid regions of the world as it plays a vital role in plant metabolism, at all growth stages. However, depending upon plant species, seed germination and seedling development could be critically affected by water stress. A high genetic variability for germination parameters was observed in recombinant inbred lines (RILs) in sunflower (Al-Charrani *et al.,* 2005).

One way to create genetic variability in cultivated sunflower is to induce mutations by irradiation with

gamma rays or chemical mutagens. Seed treatment with gamma irradiation has been extensively used to increase variability for several characteristics, such as days to flowering, seed weight, seed coat color, morphological traits and oil content in cultivated sunflower (Gupta, 1976; Robles and Lopez, 1977; Vranceanu and Stoenescu, 1982; Giriraj *et al.,* 1990; Jambhulkar and Joshua, 1999; Encheva *et al.,* 2003; Nabipour *et al.,* 2004). Mutagenesis has also been successfully used for developing variation in the fatty acid profile of sunflower (Fernández-Martinez *et al.,* 1997; Ivanov *et al.,* 1988; Osorio *et al.,* 1995; Fernández-Moya *et al.,* 2002; Ebrahimi *et al.,* 2008a).

In vitro screening methods are known to provide rapid and easy screening tests for genotypes responses to drought stress factors. Since the water potential of dry seed is very low (generally between -350 and -50 MPa) and the gradient for water uptake is large, when water is in the physiological range in the environment, germination can occur, from 0 to approximately -2 MPa (Roberts and Ellis, 1989).

There is a wide variation among species in the minimum water potential permitting growth (Evans and Etherington, 1990). According to Heydecker *et al*., (1975) polyethylene glycol (PEG) is commonly used as an osmotic-priming agent, because of its relatively inert and non-toxic nature. PEG was used for drought treatment in sunflower germination with different concentrations (Lenzi *et al*., 1995; Turhan and Baser, 2004). Genetic studies have shown a high genetic correlation between germination and grain weight in soybean (Singh *et al*., 1978).

Moreover, genetic markers have become an important tool in the selection and screening of plants for environmental stresses and for detecting genetic variability (Jahromi, 1996).

Germination and emergence characteristics are complex traits, likely to be controlled by a number of genes, and therefore, require quantitative trait loci (QTL) analysis. Identification of markers associated with important quantitative traits in a group of genotypes through multiple regression analysis offers an alternative means and has been adopted in several plant species. As an example, Vijayan *et al.,* (2006) identified several ISSR markers associated with yield traits in mulberry. Also, several AFLP markers associated with agronomic traits in 55 bread wheat genotypes were recognized by Roy *et al.,* (2006). Al-Chaarani *et al*., (2005) detected four QTLs for germination percentage and two QTLs for the time of 50% germination in sunflower in the well-watered condition. In the water stress condition, one QTL was obtained for mean germination time in *Brasica oleraceae* (Bettey *et al.,* 2000). Moreover, in recent years molecular markers have been developed for some traits, for example for high stearic and oleic acid contents (Pérez-Vich *et al.,* 2002), or for high beta- and gamma-tocopherol contents (Vera-Ruiz *et al.,* 2006; Garcia-Moreno *et al.,* 2006). The use of these markers can contribute to improve breeding efficiency. Three mutant genes were identified in *Arabidopsis thaliana* that decrease water potential for seed germination in salty or osmotic (mannitol) solutions (Saleki *et al*., 1993).

The aim of this work was to study the effect of mutagenesis in seed germination and to identify AFLP markers associated with germination parameters in sunflower mutant lines under water-stressed conditions.

MATERIALS AND METHODS

Plant material and experimental conditions

The inbred line "AS613" was developed from a cross between ENSAT-125 and ENSAT-704 by a single seed descent (SSD) programme, in our laboratory. This line was selected by Sarrafi *et al.,* (2000) for its high organogenesis response. One thousand and five hundred seeds of "AS613" were exposed to gamma rays at the Atomic Energy Center (Cadarache, France) with a dose of 75 Grays. The seeds were grown in the field and M1 plants were produced. The seeds coming from M1 plants were self-pollinated and M2 to M8 generations were advanced by self pollination through the SSD method. Between a population of about 2000 M8 lines, 30 M8 lines showing morphological differences were compared with the original line "AS613" and selected for this study. Our experiments were performed in well-watered and water-stressed conditions. A sterile germination medium containing 0.3% (w/v) Phytagel was prepared for well-water and water stress treatments. The germination medium for the water stress treatment included also 0.28% (w/v) polyethylene glycol (PEG 6000), corresponding to –1.3 MPa of water potential (Lenzi *et al.,* 1995; Foolad *et al.,* 2003). The seeds were surface-sterilized by 0.5% (v/v) calcium hypochlorite and washed three times by sterile, distilled water and briefly blotted. The seeds were then transferred to Petri dishes (95 mm diameter) containing the special media. Each Petri dish contained 20 seeds (one replication) Petri dishes were placed in a factorial design in incubators

maintained in dark where the temperature was 25°C for day (16 h) and 20°C for night (8h). The experiment consisted of a randomized block design with three replications.

Traits measurements

The number of germinated seeds was determined two times a day until the time of the percentage of germination was stable in five successive observations. The time to starting germination (TSG), the time to 50% germination (T50%G) and the time to maximum germination (TMG) were determined. The percentage of seed germination (PSG) and 1000 grain weight (1000GW) were also measured for each replication.

Genomic DNA isolation and AFLP genotyping

Genomic DNA samples of AS613 and M8 mutant lines were isolated from two-week old seedlings, according to the method of extraction and purification presented by Fulton *et al.,* (1995). Different *MseI*/*EcoRI* primer combinations were used for AFLP genotyping (Table 1). The AFLP procedure was conducted as described by Al-Chaarani *et al.,* (2004). AFLP bands were scored from the gel as presence (1) or absence (0).

Statistical analysis

The variability among the mutants for the quantitative traits was tested by ANOVA using the SAS PROC GLM (SAS Institute Inc, NC, USA). Phenotypic correlations between the quantitative traits were determined using SAS PROC CORR.

The number of polymorphic markers varied from eight for three primer combinations ("E33M61"; "E37M48"; "E38M62") to 27 for "E37M50" (Table 1). The association between AFLP markers and quantitative traits was estimated through stepwise multiple regression analysis, where each quantitative trait was considered as a dependent variable while the AFLP marker was treated as an independent variable (Vijayan *et al.,* 2006).

RESULTS

Analysis of variance of the original and mutant lines showed a highly significant variation between mutant lines for all the studied traits, except for T50%G (Table 2). The effect of water stress treatment was significant for all the critical time of germination, as well as for the percentage of seed germination. « Mutant × stress treatment » was also significant for all the traits, except for T50%G, indicating a difference among mutants in the responses of germination to drought.

In both conditions, a large genetic variation was observed among mutant lines (Table 3). The characteristics of original line 'AS613' and 5 M8 mutants presenting the highest value for the study traits are presented in (Table 3). Some mutant lines presented interesting values for more than one trait (for example M6-284-1) and some others presented interesting values just for one trait (mutant line "M6-39-2-1" for the percentage of germination in both conditions). Genetic gain (GG) calculated as the difference between the best mutant and the original line was significant for TMG and PSG under water-stressed and T50%G under well-watered conditions (Table 3).

Significant negative correlations were observed between the critical times of seed germination (TSG, T50%G and TMG) and PSG, in water-stressed condition (Table 4). A negative correlation was observed be-

Table 1. 17 AFLP primer combinations and their polymorphic markers used for genotyping M6 mutants and their original line (AS613) of sunflower.

Primer combinations	No. of polymorphic markers	Primer combinations	No. of polymorphic markers
E31M50(EAAA × MCAT)	13	E33M48(EAAG × MCAC)	9
E31M62(EAAA × MCTT)	10	E33M59(EAAG × MCTA)	15
E31M48(EAAA × MCAC)	9	E37M50(EACG × MCAT)	27
E33M50(EAAG × MCAT)	16	E37M48(EACG × MCAC)	8
E33M60(EAAG × MCTC)	11	E37M62(EACG × MCTT)	9
E33M61(EAAG × MCTG)	8	E38M62(EACT × MCTT)	8
E33M47(EAAG × MCAA)	13	E40E50(EAGC × MCAT)	9
E33M49(EAAG × MCAG)	15	E40M59(EAGC × MCTA)	9
E33M62(EAAG × MCTT)	10	Total	200

Table 2. Analysis of variance for some germination parameters in sunflower mutants. TSG: time to starting germination (h); T50%G: time to 50% germination (h); TMG: time to maximum germination (h); PSG: percentage of seed germination; ^{ns},*, **, ***: non-significant and significant at 0.05, 0.01, 0.001 probability levels, respectively.

Source	DF	Mean squares			
		TSG	T50%G	TMG	PSG
Water treatment	1	96,049.8**	280,862.4*	717,826.0***	82,133.4*
Block	2	1,011.8^{ns}	4,353.0^{ns}	91.5^{ns}	271.8^{ns}
Water treatment × Block	2	155.4^{ns}	4,909.7^{ns}	43.6^{ns}	292.6***
Mutants	29	245.7***	6,806.2^{ns}	1,274.6***	565.9***
Water treatment × Mutants	29	210.6***	6,442.6^{ns}	1,276.7***	420.5***
Error	116	70.1	5,686.4	141.5	28.4

Table 3. Seed germination traits and genetic gain (GG) of sunflower mutants under well-watered (WW) and water-stressed (WS) conditions. TSG: time to starting germination (h); T50%G: time to 50% germination (h); TMG: time to maximum germination (h); PSG: percentage of seed germination; *: significant at 0.05 level; GG: Genetic gain calculated as the differences between the best mutant and original line (AS613). Values are presented for the original line AS613 and for 4 selected mutants which present the highest values.

	TSG		T50%G		TMG		PGS	
	WW	WS	WW	WS	WW	WS	WW	WS
AS613	18	58.00	23.80	67.40	42	181.00	100.00	70.00
M6-284-1	15	58.00	18.40	70.90	39	162.00	96.67	66.67
M6-826-2	15	82.00	11.53	108.50	42	194.00	98.33	45.00
M6-143-2	18	45.00	20.80	76.40	39	175.00	100.00	70.00
M6-39-2-1	18	53.00	22.80	68.50	42	170.00	100.00	78.60
M6-39-2-2	18	53.00	21.00	74.40	39	170.00	96.66	73.33
10% best mutant	17	50.33	20.06	71.27	40	171.67	98.89	73.89
GG=best mutant-AS613	-3	-13.00	-12.27*	1.10	-3	-19.00*	0.00	8.60*

tween 1000 GW and the PSG in both conditions which became significant in water-stressed condition. In total, 40 AFLP markers associated with the germination parameters were identified in this study. Some markers are associated with more than one trait. *"E33M49-7"* marker was linked with PSG in well-watered condition as well as TSG and T50%G, in water-stressed condition. "*E33M62-10"*marker was linked with TSG and T50%G only in well-watered condition. We detected a specific marker (*E31M50-13)* for water-stressed condition associated with PSG and TSG. Some other markers were common across well-watered and water-stressed conditions, such as *E37M62-5* which was linked to PSG, TMG in water-stressed and TSG in the control condition. There were also several specific markers for each water treatment, as well as for each trait.

DISCUSSION

Phenotypic variation between genotypes

Water stress affected all germination parameters, significantly. In addition, there were differences between the mutants in term of their responses to drought. These results were in agreement with those of Turhan and Baser, (2004) and Lenzi *et al.,* (1995) in sunflower. Genotype-water stress interaction was significant for all traits,

Table 4. Correlation between some germination parameters in control (1) and drought stress (2) conditions. TSG: time to starting germination (h); T50%G: time to 50% germination (h); TMG: time to maximum germination (h); PSG: percentage of seed germination; 1000GW: 1000-grains weight (gr); *, **, ***: significant at 0.05, 0.01, 0.001 probability levels, respectively.

	Water-treatment	TSG	T50%G	TMG	PSG
T50%G	1	0.46***			
	2	0.08			
TMG	1	0.05	0.09		
	2	0.00	0.03		
PSG	1	-0.15	-0.31**	-0.01	
	2	-0.47***	-0.23*	-0.29**	
1000-GW	1	0.04	-0.09	0.07	-0.07
	2	0.09	0.12	0.20*	-0.25**

except for T50%G, suggesting that the response by a genotype in relation to other genotypes varies between the well watered and water stressed media. The significant genetic variation observed among mutant lines for the studied traits (except T50%G) revealed the efficiency of gamma-irradiation for inducing genetic variation in sunflower for seed germination parameters. Two mutant lines (M6-284-1 and M6-39-2-1) showed advantages over AS613 for TMG and PGS, respectively under water stress. These mutant lines could be used in breeding programmes to improve the ability of seed germination, under water-stressed conditions. The mutant line "M6-826-2" presented high values for T50G under the well-watered condition, compared with the original line. This mutant line also presented high values for oleic and stearic acid contents, in our previous work (Ebrahimi *et al.,* 2008b). Genetic variation was significant among mutant lines that confirmed the efficiency of gamma-irradiation for inducing genetic variation for seed germination traits.

Significant positive phenotypic correlations were found between times to starting and 50% germination (TSG and T50%G) under the well-watered condition. A similar result has been reported by Foolad *et al.,* (1998) in tomato under salt stress. The correlations among the critical times of germination indicated conceivably that similar physiological mechanisms control the times of germination (TSG and T50%G) in sunflower. Negative correlations were found between PSG with critical times of germination (TSG, T50%G and TMG) under both conditions, similar results were also observed by Al-Charrani *et al.,* (2005) in sunflower recombinant inbred lines. A significant negative correlation was observed between 1000 GW with PSG under water-stress, which is also reported by Singh *et al.,* (1978) in soybean. Torres *et al.,* (1991) suggested the importance of 1000 GW in sunflower under drought stress germination.

Marker analyses

Our results showed that some AFLP markers were associated with several traits and some others were specific for only one trait or water treatment (Table 5). The phenotypic variance explained by each marker (R^2) ranged from 12.3 to 32.3%. Multiple regression analysis has been successfully used to identify molecular markers associated with morphological and yield traits in many crops. Virk *et al*., (1996) analyzed the association between phenotypic characters in rice and specific RAPD markers. Vijayan *et al*., (2006) identified ISSR markers associated with yield traits in mulberry. In sunflower, several QTLs controlling germination parameters have been identified, using an AFLP/SSR map (Al-Chaarani *et al*., 2005). As far as we known the association between AFLP markers and germination parameter traits in sunflower mutants has not been reported previously in the literature.

Three AFLP markers (E33M62-10, E31M62-10 and E38M62-2) were detected which are common for the critical times of germination (TSG and T50%G) in the well-watered condition. "*E31M62-10"* marker was also linked to PSG under well-watered condition. Bettey *et al.,* (2000) also detected the QTL overlapping of critical times of germination in *Brassica oleracea.* This result also suggests the possibility of peliotropic effects of *E31M62-10* marker on the percentage of germination and its critical times. "*E33M50-10*" marker was also associated with both TSG and TMS under well-watered condition. We detected "*E37M48-8"* marker for T50%G under well-watered condition which was also reported for stearic, oleic and linoleic acids by Ebrahimi *et al*., (2008b). This result suggests the possible correlation between the oil content and the critical time of germination. Ujjinaiah *et al*., (1989) reported that seeds containing a higher oil percentage gave a higher germination percentage in sunflower. Lipid and protein reserves are mobilized in the germinating seed to provide carbon and nitrogen for the seedling prior to the initiation of photosynthesis (Thomas, 1993). Seed

Table 5. Main markers associated with the seed germination traits in sunflower mutants under well- watered (WW) and water-stressed (WS) conditions. TSG: time to starting germination (h); T50%G: time to 50% germination (h); TMG: time to maximum germination (h); PSG: percentage of seed germination; *, **, ***: significant at 0.05, 0.01, 0.001 probability levels; respectively. M1-M2: difference between two marker classes as revealed by the analysis of variance of trait by marker genotype.

WW					WS				
Trait	Marker	Probability	R^2 (%)	M1-M2	Trait	Marker	Probability	R^2 (%)	M1-M2
TSG	E40M59-7	*	15.00	-2.80	TSG	E33M49-7	***	32.30	-14.20
	E38M62-2	**	20.90	-2.87		E31M50-13	*	16.40	-9.53
	E37M62-5	**	19.10	2.22		E33M50-15	**	26.20	-14.27
	E33M47-8	*	16.70	2.79	T50%G	E40M50-1	**	19.80	-49.71
	E37M50-8	*	12.90	2.09		E40M50-5	**	27.90	-102.95
	E31M62-10	*	14.60	2.22		E33M49-7	*	13.60	-50.96
	E33M50-10	*	12.90	2.09	TMG	E33M59-10	**	20.40	25.54
	E33M62-10	*	14.60	2.22		E33M50-9	*	12.70	22.73
T50%G	E37M48-8	*	12.30	2.12		E33M59-7	**	21.80	-28.73
	E33M60-6	**	26.00	6.01		E33M50-3	**	21.30	27.03
	E38M62-2	*	15.70	-2.06		E33M47-10	**	20.40	25.70
	E37M62--2	*	13.40	-5.08		E37M62-5	*	12.20	-20.43
	E31M62-10	*	18.30	3.63		E33M60-8	*	14.60	22.90
	E33M62-10	**	18.30	3.64		E33M60-7	**	20.00	28.52
	E33M59-4	*	15.00	-5.21		E40M59-7	*	17.80	-29.79
TMG	E33M47-5	*	14.00	6.50	PSG	E33M62-5	*	13.00	14.88
	E33M50-26	*	14.50	3.90		E31M62-5	*	13.00	14.88
	E37M50-18	**	18.30	4.38		E37M62-5	*	15.00	-14.21
	E31M48-6	*	12.70	3.69		E31M50-13	**	20.00	15.56
	E33M50-1	*	14.50	4.10		E33M60-11	*	16.80	-15.17
	E33M50-8	*	14.50	3.94		E33M60-6	*	14.70	-15.82
	E33M50-10	**	18.30	4.38		E40M50-7	*	12.50	12.82
	E33M49-6	*	15.60	4.16					
PSG	E38M62-1	*	12.90	2.60					
	E31M50-10	*	15.00	3.05					
	E31M62-6	*	14.70	3.26					
	E31M62-10	**	23.40	-3.50					
	E33M49-7	*	15.50	3.00					

germination in oilseed plants involves the degradation of oil bodies by sequential and/or collective action of many hydrolytic enzymes such as proteases, phospholipases, lipoxygenase and lipases at different stages of lipolysis (Feussner *et al*., 1996; May *et al*., 1998; Sadeghipour and Bhatla, 2002).

We also found a non-specific marker under water-stress (*E33M49-7*) for critical times of germination. "*E33M49-7"* marker was associated with TSG and T50%G. "*E33M49-7"* marker was also associated with PSG in well-watered condition. This marker has probably a peliotropic effect on the percentage of germination, time to starting germination and the time to 50% germination.

"*E31M50-13"* marker was found to be linked to TSG and PSG and explained 20% of germination variability under water-stressed condition. The presence of this marker has an important effect on the seed germination of sunflower under water-stress condition. We also detected "*E33M60-6*" marker for PSG in the water-stressed condition. This marker *E33M60-6* was linked to the time of 50% germination in the well-watered condition, which suggested the significant correlation between T50%G and PSG. Probably "*E33M60-"6* has a peliotropic effect on PSG and the critical times of germination. "*E37M62-5"* marker was found for

critical times of germination (TSG and TMG) in both conditions (well-watered and water-stressed conditions). Also, "*E37M62-5"* was linked to PSG in the water-stressed condition. "*E37M62-5"*and*"E33M60-6"* could be used in marker assisted selection programmes for sunflower germination traits. Several specific markers were detected for each trait under both conditions, for example "*E33M59-7»* and «E33M50-3» presented a high value of phenotypic variance (21%) for TMG under water stressed condition. We detected seven markers for PSG in the water-stressed condition which presented between 12% to 20% of phenotypic variance for PSG, in water-stressed condition. Al-Chaarani *et al*., (2005) also detected four QTLs for the percentage of seed germination in sunflower, under the normal condition.

The identification of specific and non specific markers for germination-related traits, indicate that marker assisted selection may result in the development of germplasm with improved germination under normal and drought stress conditions.

REFERENCES

Al-Chaarani G., Gentzbittel L., Barrault G., Lenoble S., and Sarrafi A. (2004). The effect of gamma rays and genotypes on sunflower organogenesis traits. *Journal of Genetics and Breeding,* 58: 73-78.

Al-Chaarani G. R., Gentzbittel L., Wedzony M., and Sarrafi, A. (2005). Identification of QTLs for germination and seedling development in sunflower (*Helianthus annuus* L.). *Plant Science,* 169: 221-227.

Bettey M., Finch-savage W. E., King G. J., and Lynn, J. R. (2000). Quantitative genetic analysis of seed vigour and pre-emergence seedling growth traits in *Brassica oleracea*. *New Phytologist,* 148: 277-286.

Cook R. E. (1979). Patterns of juvenile morbidity and recruitment in plants. In: O. T. Solbrig, S. Jain, G. B. Johnson, and P. H. Raven (Eds.), *Topics in Plant Population Biology*, (pp. 207-301). Columbia University Press, Los Angeles.

Ebrahimi A., Maury P., Berger M., Shariati F., Grieu P., and Sarrafi, A. (2008a). Genetic improvement of oil quality in sunflower mutants under water stressed conditions. (ed), The17th International Sunflower Conference, (pp. 2: 509-512) Cordoba, Spain, L. Velasco.

Ebrahimi A., Maury P., Berger M., Poormohammad Kiani S., Nabipour A., Shariati F., Grieu P., and Sarrafi A. (2008b). QTL mapping of seed quality traits in sunflower recombinant inbred lines under different water regimes. *Genome*, 51: 599-615.

Encheva J., Christov M., Nenov N., Tsvetkova F., Ivanov P., Shindrova P., and Encheva V. (2003). Developing genetic variability in sunflower (*Helianthus annuus* L.) by combined use of hybridization with gamma radiation or ultrasound. *Helia,* 26: 99-108.

Evans, C. E., and Etherington, J. R. (1990). The effect of soil water potential on seed germination of some British plants. *New Phytologist,* 115: 539-548.

Fernández-Martínez J. M., Osorio J., Mancha M., and Garcés, R. (1997). Isolation of high palmitic mutants on high oleic background. *Euphytica,* 97: 113-116.

Fernández-Moya V., Martínez-Force E., and Garcés R. (2002). Temperature effect on a high stearic acid sunflower mutant. *Phytochemical*, 59: 33-38.

Feussner, I., Hause, B. Nellen, A., Wasternack, C. and Kindl, H. (1996). Lipid-body lipoxygenase is expressed in cotyledons during germination prior to other lipoxygenase forms. *Planta,* 198: 288-293.

Foolad M. R., Chen F. Q., and Lin, G. Y. (1998). RFLP mapping of QTLs conferring salt tolerance during germination in an interspecific cross of tomato. *Theoretical and Applied Genetics*, 97: 1133-1144.

Foolad M. R., Zhang L. P., and Subbiah P. (2003). Genetics of drought tolerance during seed germination in tomato: inheritance and QTL mapping. *Genome,* 46: 536-545.

Fulton T. M., Chungwognse J., and Tanksley S. D. (1995). Microprep protocol for extraction of DNA from tomato and herbaceous plants. *Plant Molecular Biology,* 13: 207-209.

García-Moreno M. J., Vera-Ruiz E. M., Fernández-Martínez J. M., Velasco L., and Pérez-Vich B. (2006). Genetic and molecular analysis of high gamma-tocopherol content in sunflower. *Crop Sciences***,** 46: 2015-2021.

Giriraj K., Hiremath S. R., and Seetharam A. (1990). Induced variability for flowering, seed weight and oil content in parental lines of sunflower hybrid BSH-1. *Indian Journal of Genetics and Plant Breeding,* 50: 1-7.

Gupta A. (1976). Differential effects of irradiation on ornamental varieties of *Helianthus annuus* L. with special reference to their cytological behavior. *Agron Lusit,* 37: 189-205.

Heydecker W., Higgins J., and Turner Y. J. (1975). Invigouration of seeds. *Seed Science and Technology,* 3: 881-888.

Ivanov P., Petakov D., Nikolova V., and Petchev E. (1988). Sunflower breeding for high palmitic acid content in the oil. 12th Int. Sunflower Conf., Novi Sad, Yugoslavia. Int. Sunflower Assoc. (pp. 463-465) Toowoomba, Australia.

Jahromi P. H. (1996). Genetic studies on salty tolerance in barley (*Hordeum vulgare* L.), PhD Thesis, The University of Reading, UK.

Jambhulkar S. J., and Joshua D. C. (1999). Induction of plant injury, chimera, chlorophyll and morphological mutations in sunflower using gamma rays. *Helia,* 22: 63-73.

Jones R. A. (1986). High salt-tolerance potential in lycoersicon species during germination. *Euphytica,* 35: 576-582.

Lenzi A., Fambrini M., Barotti S., Pugliesi C., and Vernieri, P. (1995). Seed germination and seedling growth in a wilty mutant of sunflower (*Helianthus annuus* L.): effect of abscisic acid and osmotic potential. *Environmental and Experimental Botany,* 35: 427-434.

May C., Preisig-Müller R. Höne M. Gnau P. and Kindl H. (1998). A phospholipase A2 is transiently synthesized during seed germination and localized to lipid bodies. *Biochimica et Biophysica Acta,* 1393: 267-276.

Nabipour A., Yazdi-Samadi B., and Sarrafi A. (2004). Genetic control of some morphological mutant in sunflower. *Journal of Genetics and Breeding,* 58: 157-162.

Osorio J., Fernández-Martínez J. M., Mancha M., and Garcés R. (1995). Mutant sunflower with high concentration in saturated fatty acid in the oil. *Crop Sciences*, 35: 739-742.

Pérez-Vich B., Fernández-Martinez J. M., Grondona M., Knapp S. J., and Berry S. T. (2002). Stearoyl-ACP and oleoyl-PC desaturase genes cosegregate with quantitative trait loci underlying stearic and oleic acid mutant phenotypes in sunflower. *Theoretical and Applied Genetics,* 104: 338-349.

Roberts E. H., and Ellis R. H. (1989). Water and seed survival. *Annales Botanici,* 63: 39-52.

Robles S. R., and Lopez-S E. (1977). Efecto de las irradiaciones gamma 60Co de 10 a 25 krads a la semilla de girasol (*Helianthus annuus* L.) variedad Tecmon-1. Monterrey, Invest. Inst. Technol. Estud. Super., Div. Cienc. Agropec. Maritimas. (pp. 58-60).

Roy J. K., Bandopadhyay R., Rustgi S., Balyan H. S., and Gupta P.K. (2006). Association analysis of ergonomically important traits using SSR, SAMPL and AFLP markers in bread wheat. *Current Science,* 90: 683-689.

Sadeghipour H. R., and Bhatla, S. C. (2002). Differential sensitivity of oleosins to proteolysis during oil body mobilization in sunflower seedlings. *Plant and Cell Physiology*, 43: 1117-1126.

Saleki R., Young P. G., and Lefebvre, D. D. (1993). Mutants of *Arabidopsis thaliana* capable of germination under saline conditions. *Plant Physiology*, 101: 839-845.

Sarrafi A., Kayyal H., Rachid Al-Chaarani G., Cantin F., Chaline A. S., and Durielle E. (2000). Inheritance of organogenesis parameter in cotyledons of sunflower (*Helianthus annuus* L.) *Journal of Genetics and Breeding*, 54: 227-231.

Singh C. B., Dalai M. A., and Singh. S. P. (1978). Genetic analysis of field germination in soybean. *Theoretical and Applied Genetics,* 52: 165-169.

Thomas T. L. (1993). Gene expression during plant embryogenesis and germination: an overview. *The Plant Cell,* 5: 1401-1410.

Torres M. G., Frutos S., and Duran J. M. (1991). Sunflower seed deterioration from exposure to UV-C radiation. *Environmental and Experimental Botany,* 31: 201-207.

Turhan H., and Baser I. (2004). *In vitro* and *in vivo* water stress in sunflower (*Helianthus annuus* L.). *Helia,* 27: 227-236.

Ujjinaiah U. S., Shauthamallaiah N. R. and Murali W. M. (1989). Effect of different row spacing and N and P2O5 fertilizer levels on growth, yield, yield components and quality of seed in sunflower. *Mysor Journal of Agricultural Science,* 23: 146-850.

Vera-Ruiz E. M., Velasco L., Leon A. J., Fernández-Martnez J. M., and Pérez-Vich B. (2006). Molecular tagging and genetic mapping of the *Thp1* gene controlling beta-tocopherol accumulation in sunflower. *Molecular Breeding,* 17: 291-296.

Vijayan K., Srivatsava P. P., Nair C. V., Awasthi A. K., Tikader A., Sreenivasa B., and Urs S. R. (2006). Molecular characterization and identification of markers associated with yield traits in mulberry using ISSR markers. *Plant Breeding,* 125: 298-301.

Virk, P. S., Ford-Lloyd B. V., Jackson M. T., Pooni H. S., Clemeno T. P., and Newbury H. J. (1996). Predicting quantitative variation within rice germplasm using molecular markers. *Heredity*, 27: 296-304.

Vranceanu A. V., and Stoenescu F. M. (1982). Achievements and prospects of sunflower genetics, breeding and induced mutation utilization. In: IAEA. Improvement of oil seed and industrial crops by induced mutations. Vienna, International Atomic Energy Agency.