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RESPONSE OF SUNFLOWER TO VARIOUS PRE- GERMINATION TECHNIQUES FOR BREAKING SEED DORMANCY

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Abstract

Seed dormancy is considered to be a serious constraint in sunflower seed production. Viable seeds sometimes do not germinate even in the presence of favorable environmental conditions. Such seeds are suspected to be dormant. The study was conducted under controlled/laboratory conditions during spring 2010 at National Agricultural Research Centre, Islamabad. The objective of the study was to evaluate some techniques to convert a seed from dormant to non-dormant germinable state. Dormant seeds of 21 sunflower hybrids were treated with three hot water treatments (100°/80°C) and four chemicals potassium nitrate, 0.2%, thiourea, 0.5%, ethanol, 25%, acetone, 25% for breaking seed dormancy .The untreated seed was taken as control. Soaking seeds in hot water (80°C) for 15 minutes followed by one day dry and seed treatment with acetone were found to be the most effective and successful techniques in converting the seed from dormant to non-dormant state.

Key words: Sunflower, Seed Dormancy, Genotype.

Introduction

Seed dormancy is one of the limiting factors for efficient seed production in sunflower as it delays immediate sowing of the crop. Various dormancy breaking techniques have been found to minimize the period of seed dormancy favoring maximum seed production in shortest possible time.

Sunflower is an important oilseed crop having a wide range of adaptability. It contains a high percentage of oil (40-50%) and low quantity of saturated fatty acids palmitic (4.32-9.16%) and stearic (0.20-6.64%) acids. It is a rich source of unsaturated fatty acids such as oleic acid (10.34-17.41%), linoleic acid (20.18-81.10%) and linolenic acid (0.09-20.96%) with crude protein content of 27% (Andrich *et al*., 1985; Fernandez &Alba, 1984).

Sunflower is a source of high quality edible oil. Therefore, there is a need to increase its seed production (Awais *et al*., 2013). Seed production and vigor is highly influenced by environmental factors such as temperature, water stress and depth of seed sowing. The seed shape and size also affect seedling emergence (Connor & Hall, 1997; Semerci, 2013). During the process of germination, sunflower faces the problem of seed dormancy which is one of the most important constraints to its production. Such seeds do not germinate until some particular requirement (either endogenous or exogenous) is satisfied. Dormancy is a state in which there is a block to germination due to some inherent inadequacy of the mature embryo. Generally, the sunflower seed remains dormant for more than 40 days after harvest. The seed has able to germinate about 06 days after pollination and becomes dormant again 16 days after pollination. The seed germination percentage varies with the age of developing kernel. In previous study, the highest germination percentage (97%) was observed 40 days after anthesis (DAA) and lowest (86%) for 36 (DAA) (Ahmed, 2002). The dormancy mechanism is not fully understood. However the abscisic acid (growth inhibitor) is considered to be involved in seed dormancy (Le Page-Degivery & Garello, 1991; Garello *et al*., 2000). Maiti *et al*. (2005) reported an association of dormancy with the accumulation of Abscisic acid in seeds after harvest. Other dormancy causing agents are hard seed coat and enclosing tissues as well as genotypic variability (Maiti *et al*., 2005; Subrahmanyam *et al*., 2002).

Several techniques have been adopted with little success to break dormancy including hot water treatment and the use of phytohormones. A simple technique of hydro-priming to break seed dormancy was effectively utilized to break seed dormancy in sunflower genotypes, i.e. VSF 1006, VSF 1008 and Teja. (Maiti *et al*., 2006). Various chemicals including growth regulators (gibberellic acid) have been evaluated for breaking seed dormancy in sunflower. Treatment of seed dormancy in wild sunflower with 1m M solution of gibberellic acid (GA_3) have been reported to double germination percentage over a non-treated control (Seiler, 1994). Germination of *Helianthus petiolaris* was enhanced upto 60% when treated with GA_3 (Seiler, 1998). Dormancy can also be broken down by ethylene and its precursors (Corbineau *et al*., 1990; Corbineau & Come, 2003; Oracz *et al*., 2008). Among chemicals, Ethrel is the most effective and expensive technique which is commercially employed to break seed dormancy in sunflower. The protease activity is enhanced by Ethylene resulting in the breakage of seed dormancy and successful seedling emergence (Borghetti *et al*., 2002). Several techniques have been evaluated using treatments such as growth regulators, ethrel, hydro-priming, acetone and potassium nitrate which were found to be effective in breaking seed dormancy in sunflower (Maiti *et al*., 2006). Breaking of sunflower seed dormancy upon 1% Potassium Nitrate has been reported by Mehar-un-Nisa *et al*., 2012.

It is well known fact that seed dormancy after harvest delays immediate sowing of the crop. The date of sowing has to be adjusted by seed producers to get enough time to break seed dormancy under natural conditions. There is a need for evaluation of various dormancy breaking techniques as well as genotypic response to those techniques in order to minimize the period of seed dormancy favoring maximum seed production in shortest possible time. The purpose of the study was to evaluate the effects of some pre- germination dormancy breaking techniques on sunflower genotypes.

Materials and Methods

The study conducted during Spring 2010 at National Agricultural Research Center (NARC), Islamabad. The seeds of 21 newly harvested genotypes were obtained from Oilseeds Programme, NARC, Islamabad. The genotypes and their source are mentioned as below:

S. No.	Genotypes	Origin/Country	S. No.	Genotypes	Origin/Country
1.	Hysun 33	Australia	12	AUSI GOLD 8	Australia
2.	64A-57	USA	13	AG SUN 5383	South Africa
3.	64A-41	USA	14	SA-202	Pakistan
4.	MG-2	USA	15	AUSI GOLD 62	Australia
5.	JK-CHITRA	India	16	PAN-3071	Turkey
6.	LG-54-15	France	17	AUSI GOLD 61	Australia
7.	XIPA-4	China	18	PAC-Arg-206	Argentina
8.	NOVA	Pakistan	19	M-3272	USA
9.	LG-TREGOR	France	20	M-2454	USA

The genotypes were treated with 10% sodium hypochloride (Sigma) solution to avoid disease infestation. The seeds were subjected to standard germination test (ISTA-rules) without giving any pre-germination treatment. The untreated seeds were taken as control. Seeds of each genotype were given pre-germination treatment with various chemicals and hot water. Dormancy breaking treatments were: soaking the seeds in (1) boiling water (100°C) for 15 minutes, (2) hot water (100°C) for 15 minutes followed by 01 day dry and (3) hot water (80°C) for 15 minutes followed by 01 day dry. The chemical treatments included soaking the seed in: (1) ethanol, 25% (E-Merck) for 15 minutes and rinsed with distilled water, (2) potassium nitrate, 0.2% (Riedel-de Haen, Germany) for 15 minutes, (3) thiourea, 0.5% (Riedal-de Haen, Germany) for 15 minutes and (4) acetone, 25% (E-Merck) for 15 minutes. The glassware used in this study was of analytical grade from Iwaki, Japan. The pre-germination treatments were evaluated for breaking seed dormancy after 8 days of incubation (25°C). The treated seeds along with control were subjected to standard germination test. Seeds were selected randomly. Three replications of 50 seeds each from all genotypes were subjected to germination test as described by ISTA-rules (1985). The treated seeds were rolled in wet paper and placed at 25°C in darkness in an incubator for 7 to 8 days. The germination percentage was calculated and the germination data was subjected to statistical analysis (Steel & Torrie, 1997).

Results and Discussion

Germination response of 21 sunflower genotypes to various dormancy breaking techniques is given in Table 1. The genotypes exhibited significant increase in germination percentage in response to all kinds of treatments (Table 2).

The control as well as treated seeds of all genotypes after 07 days of test exhibited significant differences in the number of germinated seed as revealed by the analysis of variance (Table 3). Among chemicals, the acetone was found to be the most effective in breaking dormancy followed by ethanol, thiourea and potassium nitrate. Adkin *et al.* (1984) reported that organic compounds with lipophilic properties are effective in breaking sunflower seed dormancy. Esen *et al*. (2009) also reported that significant citric acid effect of citric acid on germination and growth of wild cherry. Treatment with hot water (80°C) for 15 minutes followed by one day dry was more effective than other hot water treatments (Table 1). Hydro-priming for 15 and 20 hours was found to be effective in breaking seed dormancy of sunflower seeds (Maiti *et al.*, 2005). It has been reported that seed germination starts with water imbibition followed by physiological changes in the seed and is complete with the appearance of radicle (Nonogaki *et al*., 2010). Results revealed that the genotypes treated with thiourea exhibited maximum co-efficient of variation (16.03%) after control, which showed variable response of the genotypes upon treating with thiourea. The genotypes with hot water treatment (80°C + 1 day dry) exhibited the minimum co-efficient of variation indicating more or less the same response of genotypes for this treatment (Tables 2, 3). Termination of dormancy by potassium nitrate also proved effective and the germination percentage of dormant seeds in M-3272, NOVA and AGSUN 5383 increased by 40, 36 and 34%, respectively (Table 2). Thiourea was found to be more effective than potassium nitrate as dormancy breaking agent.

Table 1. Germination response of dormancy breaking techniques applied on seeds of 21 sunflower genotypes.

No.	Treatment / Dormancy breaking agents/techniques	Germination (%)
1.	Control (no treatment)	6.44
2.	Potassium nitrate (0.2%)	20.95
3.	Thiourea (0.5%)	22.28
4.	Ethanol (25%)	24.71
5.	Acetone (25%)	28.73
6.	Hot water (100°C) for 15 minutes	29.04
7.	Hot water (100°C) for 15 minutes + 1 d dry	37.9
8.	Hot water (80°C) for 15 minutes + 1 d dry	39.42

Table 2. Germination response of 21 sunflower genotypes to the dormancy breaking techniques.

No.	Genotypes	Control	Potassium nitrate (0.2%)	Thiourea (0.5%)	Ethanol (25%)	Acetone (25%)	Hotwater (100°C)	Hotwater (100°C + 1 d dry)	Hotwater (80°C)
1.	Hysun 33	3.33C	9.33 J	14.66 E-H	14.00EF	14.00I-K	17.33GH	1.33N	24.00HI
2.	64 A-57	4.00 C	17.00F-I	12.00F-H	14.00EF	10.00K	20.66FG	19.33LM	29.33G
3.	65 A-41	4.00 C	2.00 K	2.66 I	0.00H	1.33L	2.00J	2.00N	23.33I
4.	MG-2	3.33 C	24.66DE	50.66 A	36.00B	29.33F	44.66 O	24.66KL	25.33G-I
5.	JK-CHITRA	6.00C	33.33 B	50.66 A	74.00A	56.00B	3.33IJ	73.33A	64.66B
6.	LG-54-15	25.33	11.33H-J	17.33D-F	17.33DE	23.33G	17.33GH	19.33LM	20.66IJ
7.	XIPA-4	15.33	10.66IJ	8.00G-I	11.33F	14.66IJ	16.66GH	32.66J-J	10.66K
8.	NOVA	2.66 C	38.00 B	11.33F-I	30.66C	30.66EF	50.66BC	23.33LM	39.33EF
9.	LG-TREGOR	2.66 C	26.66CD	21.33C-E	5.33GH	15.33H-J	6.66IJ	50.66DE	38.00F
10.	M-3255	14.6B	21.33D-F	44.66AB	78.66A	80.66A	22.66F	60.66BC	60.66B
11.	64 S-99	5.33 C	20.66D-F	28.66C	12.33EF	12.66JK	84.66A	65.33B	43.33DE
12.	AUSI GOLD 8	2.00 C	18.66E-G	11.33F-I	40.66B	53.33B	24.00F	39.33FH	28.66GH
13.	AG SUN 5383	6.00C	36.66B	8.66F-I	30.66C	37.33C	16.66GH	31.33I-K	17.33J
14.	SA-202	2.00C	16.00F-I	10.00F-I	37.33B	34.00C-E	30.00E	37.33G-I	51.33C
15.	AUSI GOLD62	1.33 B	32.00BC	47.33A	27.33C	35.33CD	54.00B	44.00E-G	44.00DE
16.	PN-3071	15.3C	13.00G-J	16.66D-G	20.00D	36.66C	12.66H	64.00B	28.66GH
17.	AUSIGOLD-61	2.66 C	17.00F-H	24.66CD	1.33H	19.33GH	16.00GH	38.00F-I	46.00D
18.	PAC-Arg-206	1.33 C	22.00D-F	16.00E-G	26.66CD	33.33C-F	30.00E	39.33F-H	54.66C
19.	M-3272	2.66 C	44.00A	38.66B	30.66C	32.00D-F	84.66A	44.66EF	73.33A
20.	M-2454	4.00 B	17.33E-H	13.66E-H	8.66E-G	16.66H-J	48.00CD	56.66CD	77.33A
21.	PAN-7351	11.3C	7.33JK	6.00H-I	2.00H	17.33HI	17.33GH	26.00JKL	29.33A
	Mean	6.44	20.95	22.28	24.71	28.73	29.04	37.77	39.52
	LSD (%)	3.975	5.926	7.888	5.226	4.054	4.736	6.588	4.731

LSD = Least significant difference

Numbers followed by the same letters in the same column are not significantly different according to Duncan,s Multiple range Test at $p<0.05$ level

Table 3. Mean squares and Co-efficient of variation for seed germination after dormancy breaking.

Variable	DF	Control	Chemicals				Hot Water		
			Potassium nitrate	Thiourea	Ethanol	Acetone	100°C	100°C + 1 d dry	80°C
Genotype	20	119.11**	359.18**	721.67**	1341.5**	995.55**	1710.5**	1158.7**	1039.22**
Replication	2	1.78^{ns}	72.57**	8319	36.0*	9.97*	7.0^{ns}	19.1^{ns}	10.86*
Error	40								
Total	62								
CV%		27.95	12.80	16.03	9.58	6.39	7.39	7.90	5.42
F value		36.71	49.85	56.57	240.37	295.58	373.39	130.03	226.39

CV = Co-efficient of variation

The genotypes treated with 0.5% thiourea, the maximum germination response was exhibited by JK-CHITRA and MG-2 (27.3 and 26.0%, respectively) (Table 2). Reddy *et al.* (1993), Singh & Rao (1993) and Maiti *et al.* (2006) reported success in breaking seed dormancy by using potassium nitrate. Treatment of dormant seeds with certain nitrogenous compounds such as potassium nitrate and thiourea results in formation of free radicals which in turn improve seed vigor (Reddy, 1993). Tang *et al*. (2010) also stated that change in temperature also affect change in germination of *Lepidium perfoliatum* L. seeds. Exposure of sunflower achenes to ethanol and acetone results in changed permeability of cell membrane leading to permanent change which is not affected by hydration or dehydration of seed coat (Adkin *et.al.*, 1984). Hot water treatment affects the cell membrane status and results in increased membrane permeability which allows solutes and growth inhibitors to come out of the cells (Akinola *et al.*, 2000). It is important to note that the plant growth regulators are effective in minute quantities for breaking seed dormancy. Their excessive use is not recommended as it may cause toxicity and therefore produces negative effects on dormancy breaking.

During the studies it was also observed that the genotypes MG-2, M-3255, JK-CHITRA and AUSI GOLD 62 exhibited higher germination percentage than control in response to all treatments. Other genotypes responded negatively for one or more dormancy breaking treatments (Table 2), showing that germination response is genotypic

dependent. For all treatments, a low to high level of variability was observed among the genotypes as confirmed by the co-efficient of variation (Table 3). Genotypes exhibited variations in germination response upon chemical treatments for breaking seed dormancy, which may be due to difference in their genetic make-up. Other factors involved in control of dormancy are accumulation of growth inhibitors (Abscisic acid) at maturity (Le Page-Degivry & Garello, 1992) and hard seed coat (Corbineau *et al.,* 1990). At present a little success has been achieved in breaking seed dormancy since it is genotype dependent and the exact role of the dormancy breaking agents is not fully known. The dormancy breaking techniques have been successfully employed for dormancy breaking in most of the crops and in sunflower as well. Seed dormancy breaking treatments are confined up to seed germination level. Once the seed dormancy is released naturally or by pre-germination treatments (*In vitro)*, the seedling can established itself into a new plant. However, it is obvious that an understanding of the mechanism of seed dormancy and role of the factors/agents responsible for the release of dormancy may be helpful in minimizing the period of seed dormancy in sunflower after harvest.

Treatment of seeds with hot water (80°C) for 15 minutes followed by one day dry proved highly effective in breaking seed dormancy. Among various chemicals used for breaking dormancy, acetone (25%) was found to be the most effective as a dormancy breaking agent when applied to dormant seeds for 15 minutes before subjecting them to germination test. All genotypes exhibited variable response to dormancy breaking treatments showing that germination response is genotypic dependent.

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