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DEVELOPMENT AND STANDARDIZATION OF A SIMPLE TECHNIQUE FOR BREAKING SEED DORMANCY IN SUNFLOWER (*Helianthus annuus* L.)

Maiti, R.K.[1*], Vidyasagar, P.[1], Shahapur, S.C.[1],
Ghosh, S.K.[1] and Seiler, G.J.[2]

[1]*Vibha Agrotech Ltd., 501, Sirisampada, Rajbhavan Road, Samajiguda, Hyderabad- 500 082, A.P., India*
[2]*USDA-ARS, Northern Crop Science Laboratory, Fargo, ND 58105, USA*

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SUMMARY

Seed dormancy causes a great problem in efficient seed production of sunflower. Attempts have been made to develop simple techniques for breaking seed dormancy. In the first experiment, different techniques were evaluated using treatments such as a growth regulator, Ethrel, priming (water soaking followed by 2-3 days drying in shade), chemicals such as acetone (CH_3COCH_3), and potassium nitrate (KNO_3) which showed the most promise. To confirm these findings, several treatments (25% acetone, 0.2% KNO_3 and 24 h water soaking followed by drying) were further evaluated on 12 sunflower genotypes. Priming for 24 h in water is considered a simple and promising technique to break seed dormancy. This needs to be further confirmed using sunflower genotypes with different maturity groups and also with different periods of soaking in future experiments.

Key words: **sunflower, *Helianthus annuus*, dormancy, simple technique, genotypic variability**

INTRODUCTION

Sunflower is an important crop for the production of healthy edible oil that can help to reduce cardiac problems thereby increasing the demand for its oil. Therefore, there is a need to increase seed production of sunflower. Several factors such as temperature, water stress and depth of sowing greatly affect seedling emergence and seedling vigor of sunflower. The irregular shape and small seed size are other factors affecting seedling emergence (Connor and Hall, 1997). The presence of dormancy can cause great germination problems in sunflower seed. Dormancy is a temporary suspension of growth and development, which is endogenously control-

* Corresponding author: Phone: 91-40-23088078, Fax: 91-40-2332 4483,
e-mail: rkmaiti@yahoo.com

led, but also environmentally imposed (Amen, 1968). It generally takes more than 30 to 40 days after harvest for sunflower seed to attain germination capacity, thereby, delaying the immediate sowing of the crop causing a problem in its commercialization. Producers of planting seed have to manipulate the date of sowing so that they have enough time to break dormancy naturally. In one study, it was shown that seeds removed from a head 10 to 12 days after fertilization and immediately sown in moist soil have the capacity to germinate. This technique permits the sowing of the crop at several different times in a single year (Martin, 2000).

Sunflower seed attain germination capacity about 6 days after pollination and becomes dormant 16 days after pollination. Ahmed (2002) studied the effect of the age of developing kernel on germination and dormancy in a sunflower hybrid. The germination percentage obtained 40 days after anthesis (DAA) was highest (97%), followed by 34 DAA (92%), and 36DAA (86%).

The dormancy mechanism is not well known. The accumulation of abscisic acid (ABA) during maturity is considered to be involved in dormancy (Le Page-Degivry and Garello, 1991). Pericarp and seed coat thickness also contribute to dormancy. Genotypic variability in seed dormancy has been reported by Subrahmanyam *et al.* (2002). The presence of secondary dormancy and its variations among sunflower cultivars also should also be taken in account (Holec *et al.*, 2002).

Several growth regulators and chemical treatments are used to break dormancy and enhance sunflower germination. Ethylene and its precursors enhance the breaking of dormancy (Corbineau *et al.*, 1990). The synthesis of ethylene and its use in sunflower seed dormancy regulation have been discussed by Corbineau *et al.* (1990), Corbineau and Cóme (2003). In this respect, Kumari and Singh (2000) demonstrated that ethepon at a dose of 250 ppm enhanced seed germination. At present, Ethrel is used commercially to break seed dormancy in sunflower. It has been suggested that protease activity may be involved in the removal of dormancy by ethylene and the improvement of germination of the sunflower embryo (Borghetti *et al.*, 2002).

Several researchers have attempted to develop techniques to break seed dormancy but with little success. Several organic compounds such as acetone, chloroform, ethanol and many other compounds which have lipophilic properties, some degree of polarity and low molecular weight have the capacity to break seed dormancy (Adkins *et al.*, 1984; Cohn *et al.*, 1989; Corbineau *et al.*, 1991). Taylorson and Hendricks (1979) reported that ethanol breaks seed dormancy in weedy grasses. It has been stated that prolonged exposure of sunflower achene membranes to ethanol may change the cell membrane permeability leading to permanent change, not affected by hydration and dehydration. Similarly, priming in the KNO_3 solutions significantly improves germination of some crop species such as muskmelon (Nerson and Govers, 1986) and watermelon (Sachs, 1977). A 5mM KNO_3 solution doubled the germination rate of cultivated sunflower indicating that KNO_3 may influence the formation of free radicals, which in turn improve vigor

(Singh and Rao, 1993). Seiler (1993) reported that the age of achenes at harvest of wild *Helianthus annuus* and *H. petiolaris* had a significant influence on germination; the majority of germination took place by 21 days. A combination of storage temperatures and time could not break dormancy. Akinola *et al*. (2000) used different seed treatments to induce germination in wild sunflower indicating that hot water at 80°C is effective for breaking dormancy.

In the case of wild species of sunflower, Seiler (1994) reported that chemical pre-treatment (priming) of wild sunflower achenes with a 1 mM solution of gibberellic acid (GA_3) almost doubled germination percentage over a non-treated control. In a subsequent study, Seiler (1998) reported that the seeds of *H. petiolaris* treated with GA_3 enhanced germination to 60% irrespective of maturity and storage time compared with 30% in the control. It was observed that achenes harvested 20 days after flowering had greater germination than those harvested 40 days after flowering.

In the light of the reviewed literature, it was concluded that very little success has been achieved in developing a simple, efficient and inexpensive technique for breaking seed dormancy of sunflower seeds. Although the use of Ethrel is promising, it is too costly. The objective of the present paper was to select an efficient, simple, and inexpensive standardized technique for breaking seed dormancy in sunflower.

MATERIALS AND METHODS

Two experiments were conducted to select a simple, efficient, standardized, and inexpensive technique to break seed dormancy. In order to attain this objective, we used seeds of a recently harvested non-dormant sunflower genotype in the first experiment. In all experiments, standard germination tests in Petri plates, using 50 seeds per plate, were replicated four times. In order to avoid disease infestation in all the experiments, seeds were treated with a 10% sodium hypochlorite for 10 minutes, rinsed, and then were the various treatments applied.

Experiment 1

Fifty seeds of a non-dormant sunflower (VBSG–230077) recently harvested were soaked in various chemical solutions as pre-germination treatments. The treatments included: 1) control (no treatment); 2) boiling water at 100°C for 15 min; 3) boiling water at 100°C for 15 min, plus 1 day drying at room temperature; 4) hot water at 80°C for 15 min, plus 1 day drying at room temperature; 5) Ethrel soaking for 1 day in 0.3 ml/l; 6) Ethrel soaking for 1 day in 0.4 ml/l; 7) water soaking for 5 h plus 2 days drying; 8) water soaking 12 h plus 2 days drying in shade; 9) water soaking 24 h plus drying at room temperature for 7 days; 10) water soaking 34 h plus drying for 7 days at room temperature; 11) acetone (CH_3COCH_3) (25%) for 15 minute, then rinsed; 12) potassium nitrate (0.2% KNO_3) for 10 minutes.

After treatment, seeds were placed between two pieces of germination paper 9 cm in diameter in Petri dishes saturated with 15 ml of distilled water and placed at room temperature (25-30°C). All treatments were replicated four times. The number of seeds germinated was counted 4 and 7 days after treatment. A seed was considered germinated when the radicle reached a length of about 5 mm.

Experiment 2

Based on the results from the first experiment, promising treatments were further evaluated using 12 recently harvested sunflower genotypes belonging to different maturity groups based on the number of days to 50% flowering (Table 1).

Table 1: Twelve sunflower genotypes with different days to 50% flowering used to evaluate different techniques to enhance germination

No.	Genotype	Days to 50% flowering
1	VBSG-210019	62
2	VBSG-230057	59
3	VBSG-210022 (Source -1)	68
4	VBSG-230077	60
5	VBSG-230044	59
6	VBSG-230021	73
7	VBSG-230012	60
8	VBSG-210022 (Source-2)	68
9	VBSG-230064	59
10	VBSG-230065	62
11	VBSG-210002	58
12	VBSG-230029	56

Fifty seeds of each genotype were germinated in 9 cm diameter Petri dishes between two pieces of germination paper saturated with 15 ml of distilled water. The treatments were replicated four times. Petri dishes were kept at room temperature of 25-30°C in the laboratory. Four treatments were tested; 1) control (distilled water); 2) acetone 25% for 15 min; 3) 0.2 ml/l KNO_3 for 15 min; and 4) priming (24 h soaking followed by drying for 3 days at room temperature). Germination counts were taken 7 days after treatment. Statistical analysis was preformed on the total number of seeds germinated in the period of 7 days. The statistical design used was a randomized complete block design with four replications.

RESULTS AND DISCUSSION

Experiment 1

Germination percentages of seed of a dormant sunflower hybrid using different techniques to break dormancy in sunflower are given in Table 2. An analysis of var-

iance of seed treatments four and seven days after the beginning of the experiment is given in Table 3.

Table 2: Different techniques evaluated for breaking dormancy in seed of sunflower genotypes

No.	Treatment	4th day	7th day
		Germination %	
1	Control (no treatment)	96.5	99
2	Hot water 100°C for 15 min	42	83
3	Hot water 100°C for 15 min + 1day drying 20°C	72.5	96
4	Warm water 80°C for 15 min + 1day drying 20°C	86.5	92.5
5	Ethrel soaking 1 day in 0.3 ml/l	98.5	100
6	Ethrel soaking 1 day in 0.4 ml/l	99	99.5
7	Water soaking 5 h plus 2 days drying at 20°C	85	91.5
8	Water soaking 12 h plus 2 days drying at 20°C	79.5	85
9	Water soaking 24 h plus 7 days drying at 20°C	84.5	84.5
10	Water soaking 34 h plus 7 days drying at 20°C	74.5	80.5
11	Acetone 25% for 15 min	89.5	92.5
12	Potassium nitrate (0.2%) for 10 min	83	88

Table 3: Analysis of variance for seeds germinated on the 4th and 7th day using 12 different treatments

Variable	DF	F-Value			
		Seeds germinated by the 4th day		Seeds germinated by the 7th day	
Replication	3	1.15	NS	9 *	Significant
Treatment	11				
Error	33				
CV		10	5		

NS= non-significant; * signifiant $p < 0.05$; CV= coefficient of variation

Evaluation of 11 treatments seven days after treatment showed that the following were the most effective in breaking dormancy: 1) Ethrel soak for 1 day in 0.3 ml/l (100% germination); 2) Ethrel soak for 1 day in 0.4 ml/l (99%); 3) boiling water at 100°C for 15 min; (91%); 4); water soak for 5 h plus 2 day drying at room temperature (91%); 5) acetone 25% (93%), and 6) potassium nitrate 0.2 g/l (88%).

In conclusions, very little progress has been achieved by researches in breaking seed dormancy in sunflower. Seeds soaked in Ethrel (0.3 ml/l or 0.4 ml/l) showed excellent results, but the cost of the reagent is high and beyond the reach of poor farmers. However, the treatments with hot water at 80°C for 15 h, or water soaking for 5 h and 12 h produced satisfactory results which can be easily handled and which are sufficiently inexpensive to be used by poor farmers. These two techniques can be easily used in commercial planting for the production of sunflower seeds.

Experiment 2

In experiment 1, it was clear that some treatments such as soaking seeds in heated water for periods of times, acetone, and potassium nitrate increased germination percentage, but this needed to be tested further using several different genotypes. In experiment 2, 12 genotypes irrespective of age groups (days to 50% flowering) were further tested.

An analysis of variance of the seeds germinated after 7 days from 12 sunflower genotypes using three treatments is shown in Table 4. The analysis of variance indicated that there existed highly significant differences ($p<0.01$) in the number of germinated seeds among genotypes both in the acetone treatment and 24 h water priming, but no significant differences were observed in the control and in KNO_3 (Table 4).

Table 4: Analysis of variance for seed germination after seven days for 12 genotypes using four treatments

Variable	DF	F-Value			
		Control	25% Acetone	KNO_3	Priming (24 h)
Genotype	11	1.54 NS	3.66**	1.35 NS	4.40**
Replication	3				
Error	33				
CV		31	25	30	19

NS=non-significant; ** significant p<0.01; CV=coefficient of variation

The analysis of genotypic correlations indicated that germination count in the control showed highly significant positive correlation with germination count in the acetone treatment (r=0.96**), treatment with KNO_3 (r=0.87**) and also with the 24 h water priming treatment (r=0.87**). Similarly, the analysis of phenotypic correlations indicated that germination count in the control showed good correlation but at lower significance levels with the acetone treatment (r=0.79*), KNO_3 (r=0.64) and with 24 h priming (r=0.72*).

Responses of the 12 sunflower genotypes to the control, acetone, KNO_3, and 24 h water priming are shown in Table 5. It was observed that three genotypes, VBSG-230012, VBSG-230064 and VBSG-210002, showed higher germination percentage in all the treatments compared with the control. It is also observed that 24 h priming with water generally produced higher germination compared with the control, although genotypic variability in response to priming was observed.

Table 5 indicated that regardless of germination technique the genotypes showed a consistently dormant reaction across all the treatments. The genotypes which are similar in the number of days to 50% flowering have a wide variation in germination percentage. Genotypes VBSG-23OO21, VBSG-230064 and VBSG-230065 had similar germination percentages (71% for soaking 24 h), but were different in days to 50% flowering, varying from 59 to 73 days. Similarly, the genotypes which were late in maturity (66 to 73 days to 50% flowering), such as VBSG-

21OO21 and VBSG-210022 (obtained from 2 sources), showed variations in germination percentage from 17 to 71%. On the other hand, some early genotypes such as VBSG-230057 (59 days), VBSG-230012 (60 days), VBSG-210002 source 2, (58 days) and VBSG-230029 (56 days) showed large variations in germination percent, 80, 86, 61, and 29%, respectively. These facts indicate that the dormancy is highly genotype dependent. There was no correlation between days to 50% flowering and dormancy.

Table 5: Germination response of 12 sunflower genotypes to water soaking, acetone, and KNO_3 treatments

No.	Genotype	Days to 50% flowering	Control, 7 days in distilled water	Acetone treatment	KNO_3 0.2%	Water soak (24 h)
			Germination %			
1	VBSG - 210019	62	34	32	22	45
2	VBSG - 230057	**59	61	56	70	80**
3	VBSG - 210022	68	27	11	26	20
4	VBSG - 230077	60	8	10	30	16
5	VBSG - 230044	59	6	12	10	15
6	VBSG - 230021	**73	66	47	71	71
7	VBSG - 230012	60	72	85	82	86
8	VBSG - 210022	68	33	27	26	17
9	VBSG - 230064	**59	47	71	71	71
10	VBSG - 230065	62	28	23	72	71
11	VBSG - 210002	58	37	41	52	61
12	VBSG - 230029	56	21	6	24	29

In the context of the literature reviewed, it can be stated with confidence that the presence of dormancy in sunflower seeds causes great difficulties for rapid turnaround of sunflower seed for planting. The seed producers/breeders have to wait for more than 30 days to plant the seeds which have reached their optimum germination capacity. Several factors are involved in dormancy such as the accumulation of ABA in seed at maturity (LePage-Degivry and Garello, 1992) and pericarp and seed coat thickness (Corbineau *et al.*, 1990).

In the present study, acetone and potassium nitrate were used to break seed dormancy. Our findings are supported by those of other authors who used organic compounds such as acetone, chloroform, ethanol and many other compounds having lipophilic properties to break seed dormancy (Adkins *et al.*, 1984; Cohn *et al.*, 1989; Corbineau *et al.*, 1992). Singh and Rao (1993) used KNO_3 to break seed dormancy in sunflower with reasonable success.

Several researches have undertaken research to stimulate or break dormancy in sunflower. Several growth regulators to stimulate germination are commercially used to induce germination of sunflower seeds including ethylene and ethylene precursors (Corbineau *et al.*, 1990; 2003), ethepon (Kumari and Singh, 2000), and GA_3 (Seiler, 1994; 1998 in wild sunflower species). The use of growth regulators usually requires special knowledge and handling, which is beyond the capacity of most farmers in developing areas and which are too expensive to buy. In view of

these restraints, the technique developed in this study using 24 h water soak priming is very simple, easy and inexpensive and is capable of distinguishing genotypic variability for dormancy as reported by Subrahmanyam *et al.*, 2002. However, the hours of the priming period for breaking dormancy should be confirmed with another set of sunflower genotypes (a study in progress).

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REFERENCES

Adkins, S.W., Naylor, J.M., and Simpson, G.M., 1984. The physiological basis of seed dormancy in *Avena fatua*. V. Action of ethanol and other organic compounds. Physiol. Plant 62: 18-24.

Ahmad, S., Cheema, N.M., Khan, I.A., and Rana, M.A., 2002. Effect of age of developing kernel on germination and dormancy in sunflower. Pakistan J. Agric. Res. 17: 67-70.

Akinola, J.O., Larbi, A., Farinu, G.O., and Odunsi, A.A., 2000. Seed treatment methods and duration effects on germination of wild sunflower. Exp. Agric. 36: 63-69.

Amen, R.D., 1968. A model of seed dormancy. Bot. Rev. 34: 1-31.

Borghetti, F., Noda, F.N., and de Sa, C.M., 2002. Possible involvement of proteasome activity in ethylene-induced germination of dormant sunflower embryos. Brazilian J. Plant Physiol. 14: 125-131.

Cohn, M.A., Jones, K.L., Chiles, L.A. and Church, D.F., 1989. Seed dormancy in red rice. VII. Structure-activity studies of germination stimulants. Plant Physiol. 87: 879-882.

Corbineau, F., Gouble, B., Lecat, S., and Cóme, D., 1991. Stimulation of germination of dormant oat (*Avena sativa* L.) seeds by ethanol and other alcohol. Seed Sci. Res. 1: 21-28.

Corbineau, F., Bagniol, S., and Cóme, D., 1990. Sunflower (*Helianthus annuus* L.) seed dormancy and its regulation by ethylene. Isr. J. Bot. 39: 313-325.

Corbineau, F., and Cóme, D., 2003. Germination of sunflower seeds as related to ethylene synthesis and sensitivity. An overview. NATO Science Series sub-series I. Life and Behavioral Sciences 349: 216-221.

Connor, D.J., and Hall, A.J., 1997. Sunflower physiology. *In*: A.A. Schneiter (eds.) Sunflower Technology and Production. Agron. Monogr. ASA, CSSA, SSA, Madison, WI. 35: 113-182.

Holec, J., Soukup, J., and Kohout, V., 2002. Secondary dormancy in sunflower and its variation between cultivars. 12th Symposium of the European Weed Research Society, pp. 362-363.

Kumari, C.A., and Singh, B.G., 2000. Ethephon adequacy in release of innate dormancy in sunflower. Indian J. Plant Physiol. 5: 277-280.

Le Page-Degivry, M.T., Barthe, Bianco, J. and Garello, G., 1992. Involvement of absisic acid in hormonal regulation of sunflower embryo dormancy. *In*: D. Cóme, and F. Corbineau (eds.). Proc. 4th Int. Workshop Seeds, Angers, France 20-24 July, Paris pp. 615-623.

Martin, I.V., 2000. Intensive sunflower cultivation from immature seeds. Helia 23(33): 129-134.

Nerson, H., and Govers, A., 1986. Salt priming of muskmelon seeds for low temperature germination. Sci. Hortc. 28: 85-91.

Sachs, M., 1977. Priming of watermelon seeds for low-temperature germination. J. Am. Soc. Hort. Sci. 102: 175-178.

Seiler, G.J., 1993. Wild suflower species germination. Helia 16(18): 15-20.

Seiler, G.J., 1994. Dormancy and germination of wild *Heliantus* species. *In*: P.D.S. Calligan and D.J.N. Hind (eds.). *Compositae*: Biology Utilization. Proc. Intl. *Compositae* Conf. Kew, 1994, Royal Botanic Garden, Kew Vol. 2: 213-222.

Seiler, G.J., 1998. Seed maturity, storage time and temperature, and media treatment effects on germination of two wild sunflowers. Agron. J. 90: 221-226.

Singh, H.G., and Rao, G.R., 1993. Effect of chemical soaking of sunflower (*Heliantus annuus*) seed on vigour index. Indian J. Agric. Sci. 63: 232-233.
Subrahmanyam, S.V.R., Kumar, S.S., and Ranganatha, A.R.G., 2002. Genotypic differences for seed dormancy in sunflower (*Helianthus annuus* L.). Seed Res. (New Delhi) 30: 325-327.
Taylorson, R.B., and Hendricks, S.B., 1979. Overcoming dormancy in seeds with ethanol and other anaesthetics. Planta 145: 507-510.

DESARROLLO Y ESTANDARDIZACIÓN DE LA TÉCNICA SIMPLE DE ROMPER EL LETARGO DE SEMILLA DE GIRASOL (*Helianthus annuus* L.)

RESUMEN

El letargo de semilla causa grandes problemas en la producción de girasol de semilla eficaz. Se han hecho intentos de crear una técnica simple para romper el letargo de la semilla. En el primer experimento, fueron calificadas diferentes técnicas mediante el tratamiento con regulador de crecimiento, Etrel, preparación (sumergido en el agua, y luego mantenerlo en la sombra durante 2-3 días), con químicos, como acetona (CH_3COCH_3) y nitrato de potasio (KNO_3). El último de los químicos mencionados mostró los mejores resultados. Para probar estas halladas, la eficacia de varios tratamientos (25% acetona, 0.2% KNO_3 y sumergido en el agua durante 24 horas con secado) fue calificada en 12 genotipos de girasol. El sumergido en el agua durante 24 horas, fue considerado una técnica simple que promete el rompimiento del letargo de la semilla exitoso. Son necesarias unas pruebas adicionales de los resultados obtenidos, que pueden obtenerse investigando los genotipos de girasol de diferentes grupos de maduración, tanto como investigando diferentes longitudes de períodos de sumergido de semilla en el agua.

DÉVELOPPEMENT ET NORMALISATION D'UNE TECHNIQUE SIMPLE POUR ROMPRE LA DORMANCE DE LA GRAINE DE TOURNESOL (*Helianthus annuus* L.)

RÉSUMÉ

La dormance de la graine cause un grand problème pour l'efficacité de la production germinale du tournesol. Des essais ont été faits dans le but de développer des techniques simples pour rompre la dormance de la graine. Dans la première expérience, différentes techniques ont été évaluées par l'utilisation de traitements comme un régulateur de croissance, l'Ethrel, préparation (immersion dans l'eau suivie de 2 – 3 jours de séchage à l'ombre), produits chimiques comme l'acétone (CH_3COCH_3), et le nitrate de potassium (KNO_3) qui a donné les résultats les plus prometteurs. Pour confirmer ces découvertes, plusieurs traitements (25% d'acétone, 0,2% de KNO_3 et immersion dans l'eau pendant 24 heures suivie de séchage) ont été effectués et évalués chez 12 génotypes de tournesol. L'immersion dans l'eau au cours de 24 heures est considérée comme une technique simple et prometteuse pour l'interruption de la dormance de la graine. De nouvelles confirmations pouvant être obtenues par utilisation de génotypes de tournesol de différents groupes de maturité sont nécessaires ainsi que des analyses par différentes périodes d'immersion.