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EFFECT OF PRIMING WITH POTASSIUM NITRATE AND DEHUSKING ON SEED GERMINATION OF GLADIOLUS (*GLADIOLUS ALATUS*)

A. RAMZAN, I.A. HAFIZ*, T. AHMAD AND N.A. ABBASI

Department of Horticulture,
Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi, Pakistan
**Corresponding Author E- mail: decenthafiz60@yahoo.com*

Abstract

Gladiolus (*Gladiolus alatus*), belonging to the family Iridaceae is rated as the most popular flower in the world at commercial scale. The effect of different concentrations of KNO_3 (1, 2, 3, 4, 5 and 0 %) on seed germination percentage, time required for 50% germination and on mean germination time (MGT) was studied under controlled conditions. Best germination rate of 92% was achieved in T_6 (distilled water) followed by 80% in T_1 (1% KNO_3) and 70% in T_2 (2% KNO_3). Minimum time required for 50% germination i.e., 8 days was obtained with T_6 (distilled water) and in the same way shortest mean germination time required by seeds was 15 days in T_6. Bulb gained maximum weight (0.6467 g) and diameter (9.49 mm) in T_3 (3% KNO_3). Likewise, this treatment also resulted in an acquisition of 14 cm seedling length and a positive correlation was found between seedling length and growth parameters of bulb i.e., weight and diameter. In another experiment, effect of dehusking on seed germination was tested. Seed without husk gave the promising outcome of 74% germination while seeds with husk merely acquired 63% germination after 30 days.

Introduction

Gladiolus (*Gladiolus alatus*), also called sword lily belongs to the family Iridaceae. From commercial point of view, it is rated as the most popular flower in the world (Cohat, 1993). Magnificent inflorescence with variety of colors, made it attractive for use in herbaceous borders, beddings, rockeries, pots and for cut flowers (Abbasi *et al*., 2005). Gladiolus occupies 4^{th} place in International cut flower trade after rose, carnation and chrysanthemum (Farhat, 2004). However, in Pakistan it is the second most important cut flower after rose. As far as gladiolus is concerned, area under its cultivation is only 970 acre which is negligible in comparison to rose (9200) and tube rose (2787) (Khan, 2005). This constraint of small area cultivation can be overcome by increasing yield per acre that is achieveable by bringing highly productive cultivars in the field along with modern production technology package. If proper attention is paid to cut flower industry it will become our second largest export after textile (Awan, 2005).

The plants raised from seeds require four seasons to come to bloom under ordinary conditions and with best cultural conditions may be reduced to two seasons (Bose *et al.*, 2003). In recent years, a lot of work has been done on the invigoration of seeds to improve the germination rate and uniformity of growth and reduce the emergence time of some field crops (Basra *et al*., 2003) and it is highly desirable in case of breeding programs with limited number of hybrid seeds obtained from controlled or manual pollination. The beneficial effects of priming have been demonstrated in many other crops. (Mehmet *et al*., 2006) To enhance the seed germination several treatments are adopted and performance enhancements are beneficial value added techniques used on seeds after harvest, but prior to sowing (Taylor *et al*., 1998). The purpose of these treatments is to shorten the emergence period and to protect the seeds from biotic and abiotic factors during critical phase of seedling establishment so as to synchronize emergence, which lead to uniform stand and improved yield. These priming treatments which enhance seed germination include hydropriming (Afzal *et al*., 2002; Afzal *et al*., 2004), osmopriming and hormonal priming (Afzal *et al*., 2006).

The present study was conducted to enhance the germination rate of Gladiolus seeds cv. Sancerre, by using KNO_3 solution @ 1, 2, 3, 4, 5 and 0 %. Moreover, the effect of husking and dehusking was also analyzed on the germination percentage of gladiolus hybrid seeds of Sancerre, Fado and Advanced Red

Materials and Method

The present trial was conducted in the experimental area of the Department of Horticulture, Pir Mehr Ali Shah-Arid Agriculture University, Rawalpindi, Pakistan during the year 2007. Corms of three cultivars of gladiolus i.e., Sancerre, Advanced Red and Fado were imported from USA and kept for a period of three years in field by cultivating in autumn each year. At the end of spring season, the corms and cormels were removed from the soil washed, dried and treated with fungicide Dithane M-45 @ 2g per Kg of corms, then packed and stored in the cold storage at $4^{\circ}C$ till sowing. The corm were sown in September 2006 in green house and field on ridges having row to row distance 45 cm and plant to plant distance of 30 cm. DAP fertilizer was applied @ 2 bags per acre at soil bed preparation stage and two bags per acre of urea with split doses, one at the time of emergence and other about one month after emergence. Selfing of Sancerre was done by covering its spikes with butter paper bags to get seeds for priming study and hybridization of afore mentioned gladiolus cultivars was carried out to get hybrid seeds that were used in dehusking experiment.

Experiment 1

Seed priming: To accomplish the priming study, self pollinated seed of "Sancerre" were used. For early seed germination priming was done with different concentrations of potassium nitrate (KNO_3) as mentioned in Table 1. The effect of these treatments on seed germination and vigor was also determined. The percent solutions were prepared by dissolving 1, 2, 3, 4, 5 and 0 g KNO_3 per 100 ml distilled water correspondingly. Seeds were dipped in different concentrations (1 to 5%) of KNO_3 solution and in distilled water i.e., T_6 in a beaker for duration of 48 hours. For each treatment, 40 seeds were kept on filter paper treated with fungicide solution of Topsin M @ 2g/L and placed in the growth chamber at a temperature of $20 \pm 2^{\circ}C$ for germination. The optimum moisture was maintained by application of distilled water with two days interval.

The data for germination percentage (%), number of days required to attain 50% of the final germination, mean germination time (MGT) was recorded during the course of study from 20 seeds, randomly selected from each treatment. After seed germination, observation were taken for seedling length, bulb weight and bulb diameter.

Table 1. Different priming treatments to enhance seed germination of gladiolus.

Treatments	KNO_3
T_1	1 % Solution
T_2	2 % Solution
T_3	3 % Solution
T_4	4 % Solution
T_5	5 % Solution
T_6	Distilled water (0 %)
T_7	No treatment (control)

Table 2. Mean values for the effect of different concentration of KNO_3 solution on seed germination of gladiolus.

Treatments	Seed germination (%)		Mean
	After 15 days	After 30 days	
T_1 (1 % KNO_3)	68.00 g	85.00 b	80.0 a
T_2 (2 % KNO_3)	60.00 j	80.00 c	70.0 b
T_3 (3 % KNO_3)	57.00 k	78.00 d	67.5 c
T_4 (4 % KNO_3)	51.00 l	65.00 h	58.0 d
T_5 (5 % KNO_3)	63.00 i	70.00 f	66.5 c
T_6 (Distill water)	86.67 b	92.00 a	89.0 a
T_7 (Control)	47.6 m	75.00 e	61.0 b

$LSD_{0.05}$ Days = 0.015, Interaction (T×D) = 0.07, Treatments = 0.05
Means not sharing a letter differ significantly at $p<0.05$

Experiment 2

Effect of dehusking on seed germination of different gladiolus crosses: To improve the germination of gladiolus, seeds obtained after hybridization were dehusked manually and germination percentage of seeds that were with and without husk was compared from different crosses. Seeds were sown on filter paper and kept at a temperature of 20 ± 2 °C. Fungicide Topsin M @ 2g/L was applied. Seeds used were of the following crosses:
Cross 1 = Sancerre (white) V_1 × Fado (pink) V_2
Cross 2 = Advanced Red (red) V_3× Sancerre (white) V_1
Cross 3 = Sancerre (white) V_1 × Advanced Red (red) V_3

Results and Discussion

Experiment 1 seed priming

Germination percentage: The data regarding the effect of priming on germination percentage indicates significant interaction between priming treatments and number of days taken for germination at $p<0.05$ (Table 2). The most supercilious results were afforded by water treatment as compared to others, in which seeds were soaked in distilled water for 48 hours. Seed germination after 30 days was maximum (92%) with distilled water treatment (T_6) and similarly seed germination after 15 days also gave highest value of 86.67% in the same treatment. However, KNO_3 treatments did not give positive response in comparison to hydropriming. Data explicated that germination both after 15 and 30 days showed a descending trend with increasing potassium nitrate concentration from 3 to 5%.

The promotive influence of distilled water on seed germination; could be ascribed to the fact that most of the inhibitors might be washed away from the seeds (Hopkins, 1995). Consistent with our results, similar finding were observed by Harris *et al*., (2001), Arif *et al*., (2003), Sung and Chang (1993) who reported improvement in germination, reduction in germination time and enhanced emergence in hydro primed seed.

Potassium nitrate at 1% (T_1) also had a positive interaction with both time periods (15 and 30 d). The maximum seed germination obtained with this treatment was 68% and 85% after 15 days and 30 days respectively. It is plausible that its positive effect might be due to its role in influencing the permeability of the membranes which ultimately leads to activation of enzymes involved in protein synthesis and carbohydrate metabolism (Preece & Read, 1993). Most off-putting result was given up by 4% potassium nitrate treatments (T_4). Several workers have reported that KNO_3 improved the seed germination of many crops but results of this study are contrary to that and KNO_3 reduced the germination percentage. The reason might be the sensitivity of gladiolus seeds to those KNO_3 concentration used for priming. This leads to an assumption that higher concentrations exert decreasing effects on seed germination by causing death of cells and ultimately result in loss of seed viability (Nascimento, 2003). Effect of promising priming treatments on seed germination is exhibited in Fig. 1.

Time required for 50% percent germination: Statistical analysis regarding days taken to 50% germination is presented in Table 3. The difference between primed and non primed seeds for days taken to 50% germination was also significant. The non primed seed took significantly more days to 50% germination as compared to primed seed. Days taken for 50 % germination increased with increase in KNO_3 concentration from 1% to 4%. Maximum time (16 days) was taken by T_7 (control) treatment. Minimum time (8 days) was taken by T_6 (Distilled water) treatment followed by 9 days in T1 (1% KNO_3), 11 days in T_2 (2% KNO_3) and 12 days in T_5 (5% KNO_3) treatments.

The probable reason for early emergence of the hydro primed seed may be the completion of pre-germinative metabolic activities making the seed ready for radicle protrusion and the seed germinated soon after planting as compared with untreated dry seed. Emergence enhancement in KNO_3 primed seed may be attributed to metabolic repair processes, a build up of germination metabolites or osmotic adjustments during priming treatments (Bray *et al*., 1989).

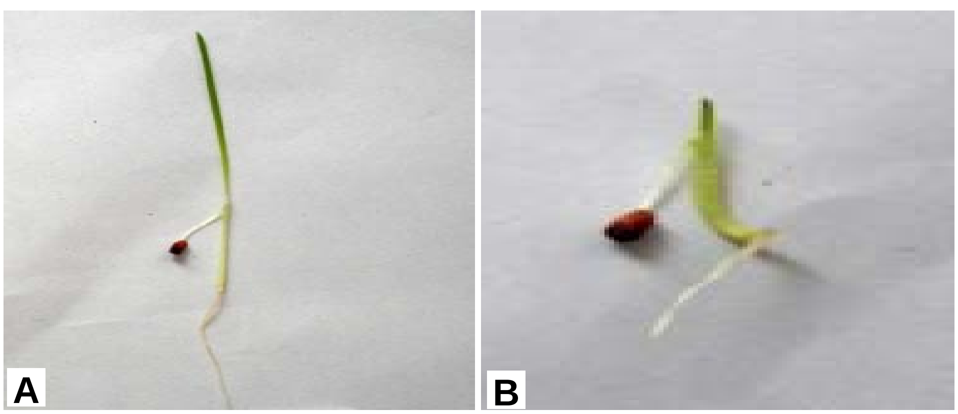

Fig. 1. Seed germination in (A) T_7 (Control) and (B) T_6 (Distilled Water)

Table 3. Effect of different concentration of KNO_3 solution on 50% germination of gladiolus seed.

Treatments	Time for 50 % Germination (DAYS)
T_1 (1 % KNO_3)	9.00 de
T_2 (2 % KNO_3)	11.33 cd
T_3 (3 % KNO_3)	13.00 bc
T_4 (4 % KNO_3)	14.00 ab
T_5 (5 % KNO_3)	12.00 bc
T_6 (Distilled water)	8.33 e
T_7 (Control)	16.00 a

Mean not sharing a letter differ significantly at $p<0.05$

Table 4. Effect of different concentration of KNO_3 on mean germination time of gladiolus seed.

Treatments	Mean Germination Time (Days)
T_1 (1 % KNO_3)	18 bc
T_2 (2 % KNO_3)	22.67 a
T_3 (13% KNO_3)	19.33 ab
T_4 (4 % KNO_3)	22.33 a
T_5 (5 % KNO_3)	21.33 ab
T_6 (Distilled water)	15.00 c
T_7 (Control)	20.33 ab

Mean not sharing a letter differ significantly at $p<0.05$

Mean germination time (MGT): Table 4 exhibits the data pertaining to mean germination time of gladiolus seed. The difference between primed and non primed seed for mean germination time (days) was statistically significant. The treatments T_2 (2% KNO_3), T_7 (control), T_4 (4% KNO_3) and T_5 (5% KNO_3) took maximum time of 20-22 days for germination. These treatments were statistically alike. Minimum time of 15 days was taken by T_6 (Distilled water) treatments followed by 18 days in T_1 (1% KNO_3) and 19 days in T_3 (3% KNO_3) treatments.

Mean germination time (MGT) was accelerated by hydropriming, without changing amount of water uptake in watermelon (Arif, 2005). Hydropriming clearly improved both rate of germination and mean germination time both under salt and drought stress conditions. Furthermore, hydropriming resulted in increase of normal germination (Basra *et al*., 2003). It is concluded that superiority of hydro priming on germination could be due to soaking time effects rather than KNO_3 treatments. The hydroprimed seeds compared to KNO_3 treated seeds were allowed to imbibe water for a longer time and went through the first stage of germination without protrusion of radicle. Our results are contrary to these findings, we have already mentioned that the seeds of gladiolus were sensitive to KNO_3 concentrations used. It showed decreasing trend in germination of the gladiolus at higher concentration. The results are suggestive to try lower concentrations for further studies.

Table 5. Effect of different concentration of KNO_3 solution on seedling length of gladiolus seeds.

Treatments	Seedling length (cm)
T_1 (1 % KNO_3)	12.45 a
T_2 (2 % KNO_3)	13.48 a
T_3 (3 % KNO_3)	14.00 a
T_4 (4 % KNO_3)	12.77 a
T_5 (5 % KNO_3)	11.03 a
T_6 (distilled water)	9.00 a
T_7 (control)	7.00 b

Mean not sharing a letter differ significantly at $p<0.05$

Seedling length (cm): Data concerning seedling length is presented in Table 5. There was a significant difference between seedling length of primed and non primed seeds. It was found that priming with KNO_3 greatly influenced the plant height as nitrogen supplied by KNO_3 is an indispensable elementary constituent of numerous organic compounds such as amino acids, proteins and nucleic acids. Moreover, it plays role in formation of protoplasm and new cells, as well as encourages plant elongation. Also, potassium is major essential element required for physiological mechanism of plant growth (Aisha *et al*., 2007). Plants of primed seed attained more length than the plants of non primed seeds. Seedling length increased with increase in concentration from 1% to 3% KNO_3 solution. Tallest plant (14 cm) were observed in T_3 (3% KNO_3) followed by 13.48 cm, 12.45 cm and 12.77 cm in T_2 (2% KNO_3), T_1 (1% KNO_3), and T_4 (4 % KNO_3) respectively. Minimum achievement of 7 cm and 9 cm was noted in T_7 (Control) and T_6 (distilled water) treatments. There was a slight difference in seedling length due to concentration of KNO_3 and increase in seedling length was observed up to 3% KNO_3 concentration then after that there was a declining trend. It may be due to availability of nitrogen and potassium from priming solution. The enhanced seedling length in primed seed may be due to the improved and faster plants seedling emergence and plant length may be due to the efficiency of the plant for utilization of nitrogen which is essential for plant growth and as well as other processes related to nitrogen metabolism (El-Bassiony, 2006).

Bulb weight (g): Analysis of variance revealed that there was a significant difference between primed and non primed seed for bulb weight as exhibited in Table 6. More bulb weight was obtained in primed seed. Maximum bulb weight (0.64g) was depicted in T_3 (3% KNO_3) followed by 0.39g in T_4 (4% KNO_3) and 0.21g in T_2 (2% KNO_3) treatment. Minimum bulb weights (0.05g), (0.08g) were noted in T_7 (control) and T_6 (Distilled water) treatments. There is positive correlation between seedling length and bulb weight as shown in Fig. 2.

Seed priming with KNO_3 might have resulted in enhancement of nutrient supply (K^+ and NO^{-3}) towards the developing seedling that results in higher weight. It has been reported that seed treatment with shikimic acid improved yield and yield components of cowpea plants by increasing the seed biomass and seed weight Aldesuquy & Ibrahim (2000). It has also been reported that matric priming resulted in higher shoot dry weight as compared with response of non treated seeds in parsley and muskmelon respectively Pill & Necker (2001) and Pill & Kilian (2000). Effect of KNO_3 on bulb yield may be due to the reason that potassium has a prevalent action in plants and is involved in maintenance of ionic balance in cell and bounds ionically to the enzyme pyruvate kinase which is essential in respiration and carbohydrate metabolism Aisha *et al*., (2007).

Table 6. Effect of different concentration of KNO_3 solution on bulb weight of gladiolus seeds.

Treatments	Bulb weight (g)
T_1 (1 % KNO_3)	0.1200 d
T_2 (2 % KNO_3)	0.2167 c
T_3 (3 % KNO_3)	0.6467 a
T_4 (4 % KNO_3)	0.3900 b
T_5 (5 % KNO_3)	0.1033 d
T_6 (Distilled water)	0.0800 d
T_7 (Control)	0.05 e

Mean not sharing a letter differ significantly at *p*<0.05

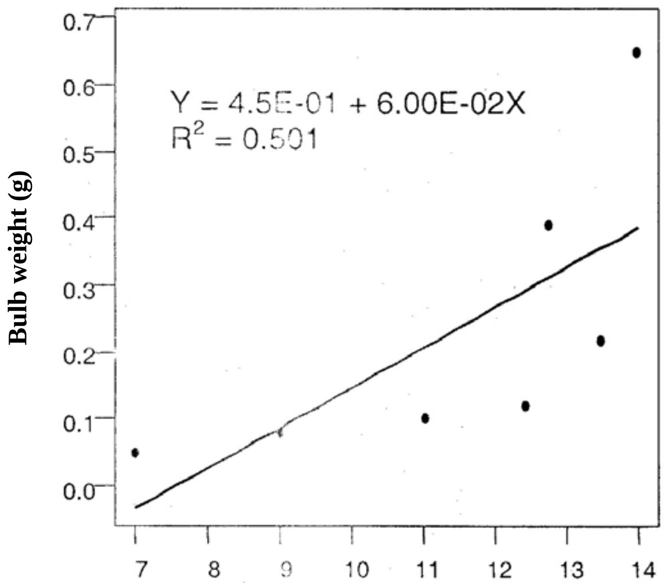

Fig. 2. Relationship between seedling length and bulb weight in gladiolus as affected by seed priming treatments.

Bulb diameter (mm): Data regarding bulb diameter is given in Table 7. The difference between primed and non primed seed was significant and bulb of primed seed gained more weight as compared to primed seed. Maximum bulb diameter 9.49 mm was noted in T_3 (3% KNO_3) treatment followed by 6.40 mm in T_4 (4% KNO_3), 6.28mm in T_5 (5% KNO_3) and 6.27 mm in T_2 (2% KNO_3). While minimum (4.40 mm) bulb diameter was observed in T_7 (control) followed by 5.42 mm in T_6 (distilled water) treatment. The results depicted positive effect of KNO_3 treatment on bulb diameter up to T_3 (3% KNO_3) and then showed declining trend. Results regarding seedling lengths and bulb diameter depicted positive effect as given in Fig. 3. Bulb diameter in T_1 (1% KNO_3) was 5.35 which was although higher than control treatment but was lower than other KNO_3 treatments of higher concentration, which can be explained by this statement that limited nitrogen supply from low concentration of potassium nitrate reduce the leaf area and leaf chlorophyll content resulting in low biomass production Zhao *et al*., (2005).

Table 7. Effect of different concentration of KNO_3 solution on bulb diameter of gladiolus seeds.

Treatments	Bulb diameter (mm)
T_1 (1 % KNO_3)	5.35 bc
T_2 (2 % KNO_3)	6.27 b
T_3 (3 % KNO_3)	9.49 a
T_4 (4 % KNO_3)	6.40 b
T_5 (5 % KNO_3)	6.28 b
T_6 (Distilled water)	5.42 bc
T_7 (Control)	4.40 c

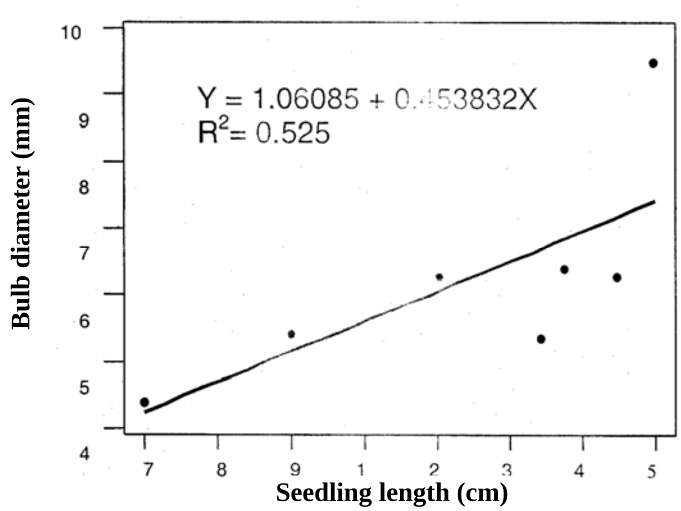

Fig 3. Relationship between seedling length and bulb diameter in gladiolus as affected by seed priming treatment.

Positive correlation between plant vigour and bulb weight implies that the seedlings which had longer length produced bulbs of large diameter. Plant height has a positive direct effect on bulb yield, diameter and weight Haydar *et al*., (2007). This increase might be due to stimulating dry mass production through enhancement of cell division and chlorophyll accumulation (Amin *et al*., 2007). The positive effect of priming (KNO_3) might be due to the availability of potassium and nitrogen that resulted in more height of seedling contributing towards bulb diameter. Positive correlation was shown in Fig. 3.

Experiment 2

Effect of dehusking on seed germination of gladiolus seed after 15 days: Results pertaining to the effect of dehusking on germination percentage of crosses of gladiolus seed are presented in Table 8. The results depicted that after 15 days the dehusked seeds gave better germination (39%) than those which were subjected to germination with husk (18%) as shown in Figs. 4 & 5. Dehusking has a pronounced effect on seed germination percentage as the efficacy of systemic seed treatment depend on the ability of applied chemical to penetrate the seed and be transported into seedling (Salanenka & Taylor, 2006). The germination frequency in case of dehusked seed was almost two time double than that of the seed with husk within a period of two weeks. It seems that dehusking increased the pace of germination particularly in case of the crosses Advanced Red × Sancerre and Sancerre × Advanced Red. However there was a little significant difference of dehusking in cross Sancerre × Fado seed.

The results indicate that the interaction of dehusking × crosses was significant ($p<0.05$) for seed germination percentage in dehusked seed. The greatest and significant advancement was observed in cross Sancerre × Fado in interaction with dehusk (46%) and husked (43%) seed. Where as minimum germination was recorded in cross Sancerre × Advance Red in interaction with dehusking (30%) and husk (5%) seed. The reason might be that husk (covering of seed) may hinder the absorption of water, exchange of gases and some time it contains inhibiting substances. Husk may not only interfere with cytokinin uptake, but may also prevent the dormancy breaking action of the plant hormone the result similar to the study of Miyoshi & Sato (1997).

Among the crosses maximum seed germination (44%) was observed in cross Sancerre × Fado and minimum (17%) seed germination was observed after 15 days in cross Sancerre × Advanced Red. This might be due to their different genetic make up or change in internal physiology of each hybrid there is difference in amount of food reserved in the seed.

Effect of dehusking on seed germination of gladiolus crosses after 30 days: Results pertaining to the effect of dehusking on germination percentage of crosses of gladiolus seed are presented in Table 9. Analysis of variance revealed that seed germination in dehusked seed was higher 74 % as compared to 63 % in with husk seed. The reason may be that seed not absorb maximum water for germination due to husk present on it. This result agree with other results that the seed coat does not impair water uptake, but play an important role in inhibiting Na+ influx into and K+ flux from the embryo to protect the seed from ion toxicity (Song *et al*., 2007).

The table also shows the interaction of dehusking with three crosses of gladiolus respectively. The results indicate that the interaction of dehusking × crosses was significant ($p<0.05$) for seed germination percentage in dehusked seed. Seed germination percentage of cross Sancerre × Fado was maximum (87%) in interaction with dehusking while there was low germination percentage (82%) in interaction with husk. Where as minimum achievement was recorded in cross Sancerre × Advance Red i.e., 50% and 33% in interaction with dehusking and with-husk seed respectively. Among the crosses the germination frequency was higher in cross Sancerre × Fado and Advanced Red × Sancerre i.e., 84% and 80% respectively. Where as minimum (41%) seed germination was observed in Sancerre × Advanced Red. Dehusking improved the germination of cross Sancerre × Advanced Red whose seeds were difficult to germinate. The reason may be internal physiology of each cross which was different and due to this carbohydrate reserved may be low in seed of cross Sancerre × Advanced Red. The data showed that dehusking improves the germination of gladiolus seed and also fasten the germination process as shown in Figs. 4 and 5.

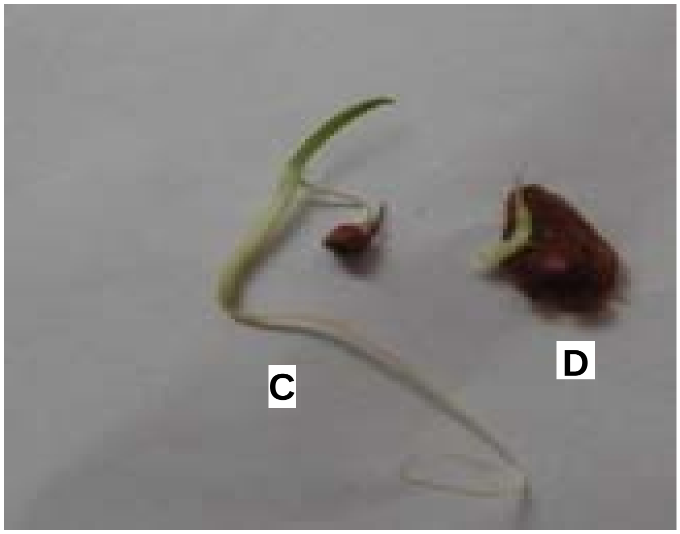

Fig. 4. Effect of dehusking (C) and with husk (D) on seed germination of cross Sancerre × Fado.

Fig. 5. Effect of dehusking (E) and with husk (F) on seed germination of cross Sancerre × Advanced Red.

Conclusion

In the present study the gladiolus seed were kept in distilled water for 48 hours. However, there is need to determine the effect of longer duration (> 48 hour) of hydration on seed germination. Present results suggest that lower concentration of KNO_3 like 0.1, 0.2, and 0.3 to 1% should be tested for priming studies of gladiolus. More over effect of other chemicals like GA_3, NaH_2PO_4 should be examined for seed germination of gladiolus.

Table 8. Effect of dehusking on seed germination of gladiolus crosses after 15 days.

Crosses	Seed germination % after 15 days		Mean
	Dehusked	With husk	
Sancerre × Fado	46 a	43 b	44 a
Advanced red × Sancerre	30 d	5 f	17 c
Sancerre × Advanced red	41 c	7 e	24 b
Mean	39 a	18 b	

$LSD_{0.05}$ Treatments = 1.027, Interaction (T×C) = 1.77, Crosses = 1.258
Mean not sharing a letter differ significantly at $p<0.05$

Table 9. Effect of dehusking on seed germination of gladiolus crosses after 30 days.

Crosses	Seed germination % After 30 Days		Mean
	Dehusked	With husk	
Sancerre × Fado	87 a	82 c	84 a
Advanced Red × Sancerre	85 b	76 d	80 b
Sancerre ×Advanced Red	50 e	33 f	41 c
Mean	74 a	63 b	

$LSD_{0.05}$ Treatments = 1.027, Interaction (T×C) = 1.77, Crosses = 1.258
Mean not sharing a letter differ significantly at $p<0.05$

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