INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2003/05–4–499–503
http://www.ijab.org

# Effect of Seed Humidification on Germinability, Vigor and Leakage in Cockscomb (*Celosia argentea var. cristata* L.)

M. MUMTAZ KHAN[1], M. QASIM, M. JAVED IQBAL, AFZAL NAEEM AND M. ABBAS
*Institute of Horticultural Sciences, University of Agriculture, Faisalabad–38040, Pakistan*
[1]Correspondence: mumtaz59pk@hotmail.com

## ABSTRACT

The effects of humidification, considered to be rapidly increased seed germinability and vigor in cockscomb (*Celosia argentea var. cristata* L.) seeds, were explored. Seed germination percentage, vigor, germination speed and seed content leakage were studied in relation to humidification process. Humidification greatly increased the final germination percentage, germination speed and decreased the time taken to 50% germination. Stabilization in seed content leakage was associated with the increased membrane repair mechanism. Germination percentage was increased upto 100%, after humidification treatment, and germination speed increased significantly ($P < 0.05$) from 23.16 (7 days priming) to 31.06 germinated seeds per unit day (control). Seed content leakage remained unchanged even after 7 days of humidification. Increased germinability and seedling vigor were associated with the function of membrane repair mechanism in seed cells.

**Key Words:** Cockscomb; Humidification; Priming; Seed germination; Seed content leakage; Repair mechanism

## INTRODUCTION

Cockscomb (*Celosia argentea var. cristata* L.) is among the most popular summer flowers, and considered an ideal for garden display. Successful cultivation of the flower depends on quality of seeds such as viability, vigor and storage potential etc. The deterioration is common physiological process during prolonged storage of seeds, wherein seed membrane degradation and accumulation of inhibitors occurs, resulting in low field emergence and loss of viability. Seed germination can be enhanced with several treatments such as humidification (Finnerty *et al.,* 1992; Pijlen *et al.,* 1996), hydro-priming (Rudrapal & Nakamura, 1988), osmotic priming (Dell'Aquilla & Tritto, 1990) and alternate hydration-dehydration (Nath *et al.,* 1991). Many workers have attempted to increase the seed germination capacity and vigor by using different priming treatments in various field crops (Brocklehurst & Dearman, 1983; Rao, *et al.,* 1987; Bray *et al.,* 1989; Sivritepe & Dourado, 1995; Harris, 1996; Lee & Kim, 1999).

Once sown, seeds spend significant amount of time to absorb water from the soil, by reducing this time seeds can be made ready to germinate and seedlings emerge more quickly. The easiest possible way to achieve this is the seed priming treatment. Seed priming or preplant seed treatments for example osmoconditioning, matriconditioning, rehydration and humidification can be employed to reduce the duration required to germinate, to synchronize germination and to improve the germination rate in laboratory and field emergence of low quality seeds (Khan, 1992). So humidification can be used as priming tool because any treatment which arrests seed germination could possibly be used as priming treatment (Sivritepe & Dourado, 1995).

Priming involve a period of controlled hydration of the seeds to a point closed to, but before the emergence of the radicle after which seeds are dried back to their initial moisture content before sowing (Khan, 1992; Basu, 1994), whereas humidification and aerated hydration differ from priming in humidity density and specific duration (Thronton & Powell, 1992; Pijlen *et al.,* 1996). Humidification is a pre-sowing hydration treatment in which seeds are equilibrated under conditions of high humidity (Finnerty *et al.,* 1992; Pijlen *et al.,* 1996; Suzuki & Khan, 2001).

Although the mechanism of pre-plant seed treatments is not fully understood, it has been observed that the physiological and biochemical changes occur during the seed treatment. The compound starch grains in the endosperm disintegrate into tiny starch granules and α-amylase activity of the seeds was extremely high after priming treatment (Lee & Kim, 1999). It could be allowed for seeds to develop the germination sequence immediately before germination. This would be the basis for germination improvement of the primed seeds (Lee & Kim, 1999). In most of the cases seeds have lost their quality when they reach to the farmer but primed seeds may show increased final germination percentage. The priming conditions had a large influence on emergence and seedling vigor in sorghum and argued that germination speed was an important determinant of successful seedling establishment (Harris, 1996). Rapidly germinating seedlings could also produce deep root system and better seedling establishment in many field crops.

These studies were initiated to explore the effects of saturated humidification at high temperature (45$^{o}$C, to obtain better moisture absorption rate) on seed germinability and vigor (if any) in cockscomb (*Celosia argentea var. cristata* L.) seeds.

## MATERIALS AND METHODS

**Seed material.** Experiments were conducted on six months old, round and equal sized cockscomb (*Celosia argentea var. cristata* L.) seeds. The seeds lots were obtained from the collections of the Institute of Horticultural Sciences, University of Agriculture, Faisalabad. Moisture content of seed samples was recorded (ISTA, 1993). Seeds were surface sterilized with 5% sodium hypochlorite solution for 10 minutes and rinsed with double distilled autoclaved water (Mumford & Grout, 1979). The seeds were dried back at 25°C±1 and stored in aluminum foil bags at 5°C until use.

**Seed humidification treatment.** Three lots of seed were subjected to high humidification (100% R.H.) at 45°C (to obtain better moisture absorption rate) for 3, 5 and 7 days in a controlled chamber (Plant Growth Chamber, VINDON, England). Following the priming (humidification) treatment, seeds were removed according to predetermined intervals and the moisture content was recorded. The seeds were air dried at 25°C±1 under shade until their original moisture content was restored (Basra *et al.,* 2002).

**Germinability and vigor tests.** Germination was observed daily at 23°C±2 according to ISTA (1993) and performed on five replicates of 25 seeds each. The seeds were incubated on top of moist Whatman No. 1 double filter papers in 9 cm Petri dishes at 23°C±2 in growth chamber under white fluorescent light. Water availability was checked daily and topped-up as necessary. The seedling radicle length was recorded at 23°C±2 after every 48 h time interval. The percentage germination and seed germination speed at 23°C±2 after every 24 h were calculated by the following formula derived by Kotowski (1926).

$$\text{Germination speed} = \frac{\sum n}{\sum (n \times Dn)} \times 100$$

Where, n = number of seedlings germinated on day (D).
Dn = number of days from sowing to corresponding to n.

The time to get 50% germination (T50) was calculated by following formula proposed by Coolbear *et al.* (1984) and physically worked out in Fig. 4

$$T_{50} = t_i + \frac{(N+1)/2 + n_i}{(n_j - n_i)}(t_j - t_i)$$

**Seed content leakage.** Membrane permeability of treated and untreated seeds was studied by recording the electrical conductivity of seed leachates following the method of Khan *et al.* (2003). Twenty replications of single seed from each treatment were soaked in 0.5 mL deionized water for 24 h at 25°C±1. The electrical conductivity of seed-steep water was recorded by using digital Nikon EC meter. All the observations were made after 3, 6, 12, 18 and 24 h of soaking in water.

## RESULTS AND DISCUSSION

**Moisture content.** The moisture content of the cockscomb seeds rose to 13.20% in 7 day primed seeds compared to control (5.86%). While the moisture contents of 3 day and 5 day treatments were 6 and 8.92%, respectively. This increase in the seed moisture content was possibly due to the moisture absorption from the saturated atmosphere (100% R.H.).

**Mean germination percentage.** Germination percentage was increased upto 100% after 7 days of priming treatment. There was a linear relationship between priming treatment and final germination percentage. It was 78% in control and 86, 89 and 100% in 3, 5 and 7 day treatments, respectively (Fig. 1). Similar findings were recorded in pea seeds by Sivritepe and Dourado (1995). This increase in the germination percentage may be attributed due to RNA and protein metabolism as enhanced by priming (Khan *et al.,* 1978). So priming can be prerequisite to increase seed germinability (Hurly *et al.,* 1989). The priming treatment showed significant (P < 0.05) effect on seed germination percentage as compared to control.

**Seedling vigor.** Seed radicle length was measured to determine the seedling vigor. After every 48 h of germination, radicle length was recorded as described by Khan *et al.* (2003). Radicle length was significantly (P< 0.05) increased after 7 day of priming. It increased upto 3.2 cm in 7 day treatment compared to 2.51 cm (control) while the radicle lengths of 3 and 5 day treated germinating seeds were 2.79 and 3.1 cm, respectively (Fig. 2). The radicles of properly primed germinating seeds have shown faster growth than non-primed germinating seeds in rice seeds (Lee & Kim, 1999). So it may be postulated that priming has increased the vigor of cockscomb seedlings. Sivritepe and Dourado (1995) stated that changes in the activities of such enzymes as esterase, phosphatase and 3-phospho-glyceraldehyde dehydrogenase suggest that metabolism of seed storage materials as carbohydrates, fats and proteins may underlie the increased germination and vigor induced

**Fig. 1: Effect of artificial priming on germination percentage of *Celosia argentia* seeds**

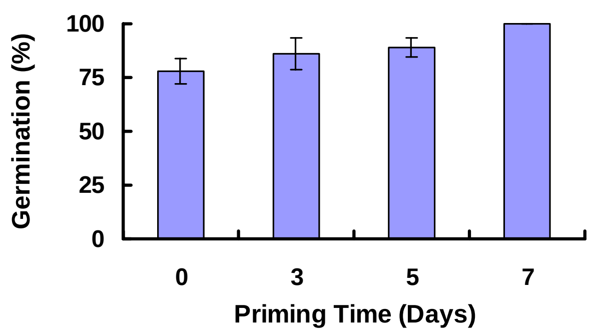


**Fig. 2. Effect of artificial priming on the radicle length of *Celocia argentia***

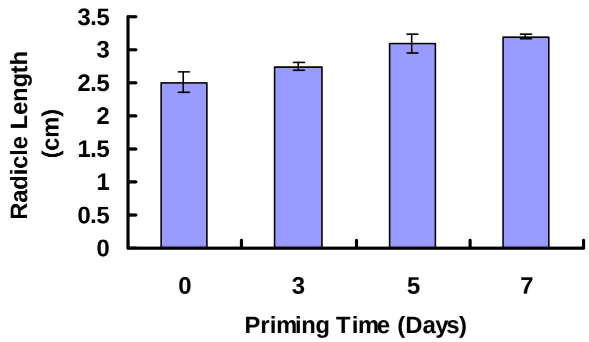
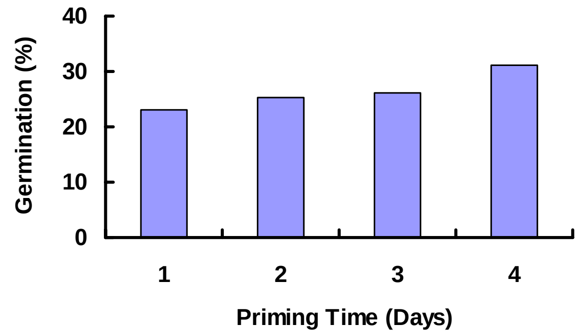



**Fig. 3. Effect of artificial priming on germination speed of *Celocia argentia* seeds**




by priming.

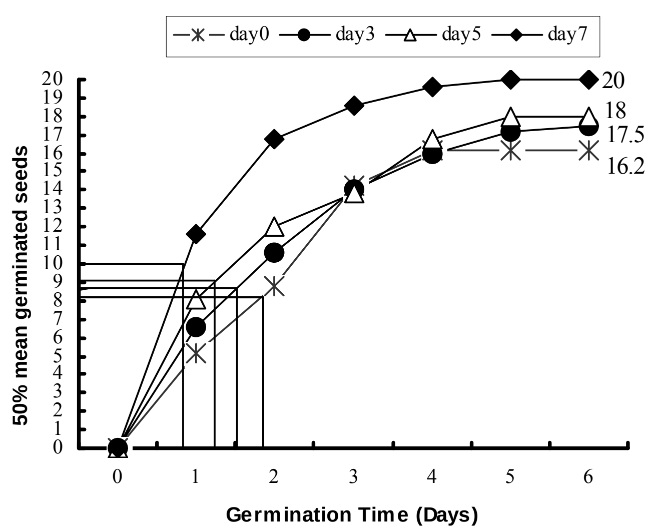
**Germination speed.** Germination speed is a direct measure of seed vigor. Priming treatment increased the germination speed significantly ($P<0.05$); it means larger number of seeds were germinated per unit day compared to the control treatment. Results showed that the germination speed of 7 day was the fastest (31.06 germinated seeds per unit day) and that of control was the lowest (23.16 germinated seeds per unit day) (Fig. 3). This indicates that priming has positive effect on speeding up the germination process. Germination speed of 3 and 5 day primed seeds were also directly proportional to the priming period, it was 25.40 and 26.13 germinated seeds per unit day for 3 and 5 day of priming treatment, respectively. Vigorous seed lots have more germination speed compared to non vigorous seeds (Khan *et al*., 2003). These results are supported by the earlier findings of Sivritepe and Dourado (1995) in pea seeds.

**Time to get 50% germination (T50).** Results showed that all priming treatments significantly ($P<0.05$) reduced the time to reach 50% of the total germination (Fig. 4). The fastest rate of germination (calculated as time to get 50% germination) was observed in the 7 day treatment (0.75 days) followed by 5 (1.2 days) and 3 day treatments (1.5 days). The T50 value of the control (non-primed seeds) was the highest (1.7 days). Similar findings were previously described by Simak (1976), Thanos *et al*. (1989) and Sivritepe and Dourado (1995) who described that priming treatments increased the final germination and the rate of germination i.e. decreased the germination time. Lee *et al.* (1998) stated that time to obtain 50% germination level and uniform germination decreased upto 4 days when primed. A faster T50 was observed in rice seeds after priming treatment (Lee & Kim, 1999).

**Seed content leakage (EC).** Membrane permeability of treated and untreated seeds was studied by recording the electrical conductivity of the seed leachates after 3, 6, 12, 18 and 24 h of soaking in deionized water. The EC of seed-steep water remained constant (14.52 mS/cm) after 24 h observations, even after 7 day treatment. Although there was a significant increase in the seed content leakage in relation to soaking period in deionized water but it remained stable in every treatment corresponding to each soaking period. The seed content leakage after 24 h soaking period was 14.52 mS/cm in all priming treatments and for 18 and 12 h were 12.72 and 10.04 mS/cm, respectively (Fig. 5). This shows that EC increased with the passage of soaking time but not affected by priming treatment. These findings are in accordance with Basra *et al.* (2002). It means seed membrane damage may be repaired during humidification treatments that stimulated the germination percentage and developed its vigor.

Biochemical studies on seeds showed that RNA and protein metabolism are enhanced by priming suggesting that this treatment makes available precursors utilized for macromolecular synthesis (Khan *et al.,* 1978). Cytological examinations after seed priming showed decreased

**Fig. 4. Effect of humidification on time taken to 50% germination**



frequency of visible chromosomal aberrations whilst the increased percentage of germination (Sivritepe & Dourado, 1995). The α-amylase activity was higher in primed rice seeds compared to non-primed seeds. This higher α-amylase activity of primed seeds may be related to the higher sugar content (Lee & Kim, 1999). All such biochemical studies have demonstrated that enzyme activities are enhanced after priming and this suggests that there was no degradation in seed components. This strengthens our findings on seed electrical conductivity which remained constant throughout experiment.

Stabilization in seed content leakage in cockscomb seed is possibly associated with the increased membrane repair mechanism. Seed content leakage remained unchanged even after 7 days of priming. Increased germinability and development of vigor were associated with the function of membrane repair mechanism in cells. Priming has been identified as a possible treatment which arrests germination and facilitates repair of cell membranes damages. The relative effectiveness of the priming treatment in restoring the vigor of stored seeds is expressed by increased germination, as previously reported Dell'Aquila *et al*. (1984). In general, fast germination is accompanied by high protein synthesis in germinating embryos (Sivritepe & Dourado, 1995). Roberts (1981) has sproposed that the repair system in lettuce may be operative at seed moisture content as low as 16%. This moisture content is easily attained by moisture equilibrium of lettuce seeds with a water saturated atmosphere i.e. 100% humidity (Basu *et al*., 1979). Evidence that the deleterious effects of storage in seeds may be reversed during subsequent priming (rehydration) has been reported previously (Villiers & Edgcumbe, 1975).

In conclusion, the results of the present study suggest that priming of cockscomb seeds at 100% relative humidity level at 45$^{o}$C is a better treatment to increase the percentage germination, germination speed and decrease the time to get 50% germination. The results also suggest that this led to possible repair of cell membrane damage during treatment. But further work is needed to ascertain that what and when the repair takes place and what is the best duration of priming for cockscomb seeds to further enhance the germination speed, T50 and cell membrane repair mechanisms. The present study confirms previous suggestion made by Rao (1986), working with lettuce, that priming treatments before sowing seeds for regeneration accessions should be recommended as a general practice in gene banks (especially when the seed has gone considerable storage). This treatment is not only useful for regeneration purposes but also reduces the incidence of heritable genetic damage which is manifested as mutational physical expressions.

## ACKNOWLEDGEMENT

We are thankful to the University of Agriculture, Faisalabad for providing funds through Research Promotion Fund Programme to carry out these studies.

## REFERENCES

Basra, S.M.A., M.N. Zia, T. Mehmood, I. Afzal and A. Khaliq, 2002. Comparison of different invigoration techniques in wheat (*Triticum aestivum* L.) seeds. *Pakistan J. Arid Agric.,* 5: 11–6

Basu, R.N., D. Pan and B. Punjabi, 1979. Control of lettuce seed deterioration. *Indian Plant Physiol.,* 22: 247–53

Basu, R.N., 1994. An appraisal of research on wet and dry physiology seed treatments. *Seed Sci. & Technol.,* 22: 107–26

Bray, C.M., P.A. Davison, M. Ashraf and R.M. Taylor, 1989. Biochemical changes during osmopriming of leek seeds. *Ann. Bot.,* 63: 185–93

Brocklehurst, P.A. and J. Dearman, 1983. Interactions between seed priming treatments and nine seed lots of carrot, celery and onion. I. Laboratory germination. *Ann. Appl. Biol.,* 102: 577–84

Coolbear, P., A. Francis and D. Grierson, 1984. The effect of low temperature pre–sowing treatment on the germination performance and membrane integrity of artificially aged tomato seeds. *J. Exp. Bot.,* 35: 1609–17

Dell'Aquila, A., D. Pignone and G. Carella, 1984. Polyethylene glycol 6000 priming effect on germination of aged wheat seed lots. *Biol. Plant., (Praha),* 14: 166–73

Dell'Aquila, A. and V. Tritto, 1990. Ageing and osmotic priming in wheat seeds: Effects upon certain components of seed quality. *Ann. Bot.,* 65: 21–6

Finnerty, T.L., J.M. Zajicek and M.A. Hussey, 1992. Use of seed priming to by pass stratification requirements of three *Aquilegia* species. *Hort. Sci.,* 27: 310–13

Harris, D., 1996. The effects of manure, genotype, seed priming, depth and date of sowing on the emergence and early growth of *Sorghum bicolar* L. Moench in semi–arid Botswana. *Soil Tillage Res.,* 40: 73–88

Hurly, R.F., J. Van Staden and M.T. Smith, 1989. Guayule (*Parthenium argentatum* Gray) seed germinayion. The effect of water soaks, sodium hypochlorite, gibberellic acid and gibberellin4/7 applied as seed pre–treatments. *Seed Sci. Technol.,* 17: 223–33

ISTA, 1993. International rules for seed testing. *Seed Sci. Technol.,* 21**:** 1–288

Kotowski, F., 1926. Temperature relations to germination of vegetable seeds. *Proc. American Soc. Hort. Sci.,* 23: 176–84

Khan, A.A., K.L. Tao, J.S. Knypl, B. Borkowska and L.E. Powell, 1978. Osmotic conditioning of seed: physiological and biochemical changes. *Acta Horticulturae,* 83: 267–78

Khan, A.A., 1992. Preplant physiological conditioning*. Hort. Review,* 13: 131–81

Khan, M.M., M.J. Iqbal, M. Abbas and M. Usman, 2003. Effect of accelerated ageing on viability, vigor and chromosomal damage in pea (*Pisum sativum* L.) seeds*. Pakistan J. Agri. Sci.,* 40: 50–4

Lee, S.S., J.H. Kim, S.B. Hong, M.K. Kim and E.H. Park, 1998. Optimum water potential, temperature and duration for priming of rice seeds. *Korean J. Crop Sci.,* 4: 1–5

Lee, S.S. and J.H. Kim, 1999. Morphological change, sugar content and α–amylase activity of rice seeds under various priming conditions. *Korean J. Crop Sci.,* 44: 138–42

Mumford, P.M and B.W.W. Grout, 1979. Desiccation and low temperature (-196$^{o}$C) tolerance of *Citrus limon* seed. *Seed Sci. Technol.,* 7: 407–10

Nath, S., P. Coolbear and J.G. Hampton, 1991. Hydration–dehydration treatments to protect or repair stored "Karamu" wheat seeds. *Crop Sci.,* 3: 822–6

Pijilen, V.J.G., S.P.C. Groot and H.L. Kraat, 1996. Bergervoet on germination performance, moisture content and DNA synthesis. *Seed Sci. Res.,* 6: 57–63

Rao, N.K., 1986. Chromosomal aberrations and seed mutatioms induced in lettuce (*Lactuca sativa* L.) seeds during storage. *Ph. D. Thesis,* Reading University, UK.

Rao, N.K., E.H. Roberts and R.H. Ellis, 1987. The influence of pre and post-storage hydration treatments on chromosomal aberrations, seedling abnormalities and viability of lettuce seeds. *Ann. Bot.,* 60: 97–108

Roberts, E.H., 1981. Physiology of ageing and its application to drying and storage. *Seed Sci. Technol.,* 9: 359–72

Rudrapal, D. and S. Nakamura, 1988. The effect of hydration–dehydration pretreatment on egg plant and radish seed viability and vigor. *Seed Sci. Technol.,* 16: 123–30

Simak, M., 1976. Germination improvement of scots pine seeds from circumpolar regions using polyethylene glycol. In: Hatano K., S. Asakawa, M. Katsuta, S. Sasali, T. Yoloyama, (eds.) *Proc. the Second Int. Symp. on Physiol. Seed germination,* Fuji, Tokyo: Government Forest Experiment Statio., 145–53

Sivritepe, H.O. and A.M. Dourado, 1995. The effects of priming treatments on the viability and accumulation of chromosomal damage in aged pea seeds. *Ann. Bot.,* 75: 165–71

Suzuki, H. and A.A. Khan, 2001. Effective temperatures and duration fro seed humidification in snap bean (*Phaseolus vulgaris* L.). *Seed Sci. Technol.,* 28: 381–9

Thanos, C.A., K. Georghiou and H.C. Passam, 1989. Osmoconditioning and ageing of pepper seeds during storage. *Ann. Bot.,* 63: 65–9

Thronton, J.M. and A.A. Powell, 1992. Short term aerated hydration for the improvement of seed quality in *Brassica oleracea. Seed Sci. Res.,* 2: 41–9

Villiers, T.A. and D.J. Edgcumbe, 1975. On the cause of seed deterioration in dry storage. *Seed Sci. Technol.,* 3: 761–74

**(Received 01 June 2003; Accepted 10 September 2003)**