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DOES PRIMING PROMOTE GERMINATION AND EARLY STAND ESTABLISHMENT OF FRENCH MARIGOLD (*Tagetes patula* L.) SEEDS BY INDUCING PHYSIOLOGICAL AND BIOCHEMICAL CHANGES?

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Abstract. Poor plant stand establishment is a common problem for marigold production. Exposure to seed priming is one of the strategies being used to accelerate the activation of plant growth, especially seed germination in many crops. Therefore, an experiment was conducted to investigate the influence of different seed priming techniques on performance of French marigold. For this purpose, different seed priming techniques: hydroprimng, 50 mM $CaCl_2$, 100 mg L^{-1} salicylic acid and 100 mg L^{-1} ascorbic acid were assessed. Seeds without any treatment were considered as control. Priming with 50 mM $CaCl_2$ was proved to be the most effective treatment in enhancing germination and seedling vigour among all other treatments including control as shown by higher final germination/emergence, germination energy and lower mean germination/emergence time. Furthermore, positive correlation between reducing sugars and dry weight and α-amylase activity and the concentration of reducing sugars indicates that enhancement due to priming could be attributed to higher reducing and total sugars as well as higher α-amylase activity in primed seeds.

Key words: germination, priming, marigold, seedling vigour, stand establishment

INTRODUCTION

Marigold (*Tagetes* spp.) is widely used for beautification and as landscape plants due to its ideal height and attractive colour. It is ideal for rockeries, edging, hanging baskets and in window boxes. The species is grown around field crops to control pest activity [Tereschuk et al. 1997]. Poultry industry is extensively using marigold petals as a natural source of xanthophylls pigments to strengthen yellow colour of egg yolk [Kaul et al. 2000]. The oil of marigold species is highly used in high quality perfumes and also

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carotenoid pigments are used in food industry [Vasudevan et al. 1997]. Caroteniod pigments have beneficial role for treatment of skin tumor, dermatological diseases and cancer in human being [Bosma et al. 2003a]. Thus, cultivation of marigold as an alternative crop is gaining popularity day by day in agriculture business due to the commercial use of carotenoids in pharmaceutical and poultry industries.

One of the greatest problems for marigold production is poor plant stand establishment under hot or cool field conditions [Bosma et al. 2003a]. Direct sowing is more economical for the producer than using transplants. Labour costs are reduced, especially if a precision seeder is used, and there are no propagation and transplant production expenses [Bosma et al. 2003b]. Though direct sowing results in uneven germination, slow emergence, and poor stand establishment still it is less expensive initially and therefore widely used in field operations.

Seed priming is simple and cost effective pre-sowing treatment which improves germination and synchronizes earlier and vigorous stand establishment in agricultural and horticultural crops [Afzal et al. 2002, Mirshekari 2012]. Seed priming is a hydration followed by redrying process for limited time which allows all pregerminative metabolic processes but prevents radicle emergence [Bradford 1986]. Moreover, primed seeds germinate in a wider range of temperatures and are less sensitive to oxygen deprivation than unprimed ones [Corbineau et al. 1993]. Priming may be used to enhance germination of direct-seeded plants, increase seedling emergence uniformity under adverse environmental conditions, and improve stand establishment.

Limited work has been reported regarding physiological and biochemical aspects of French marigold seed enhancements. Therefore, this study was carried out to investigate the role of various seed priming techniques in enhancing germination and seedling vigour of marigold. The study was carried out to investigate the effect of seed priming on enhancing germination and seedling vigour of marigold species and to explore the possible biochemical basis of this enhancement.

MATERIALS AND METHODS

The seeds of French marigold (*Tagetes patula* L.) were obtained from Evergreen Nursery, Faisalabad, Pakistan. Healthy seeds were selected and used for the experiments. All damaged seeds were removed.

Seed priming protocols. For all seed priming treatments 5 g of seed sample was used. The ratio of seed and working solution was kept 1:5 (g ml^{-1}). Seeds of French marigold were soaked in aerated solution of 50 mM solution of $CaCl_2$, 100 mg l^{-1} salicylic acid, 100 mg l^{-1} ascorbate and distilled water for 24 h at 25 ± 2°C. After priming, seeds were quickly rinsed with distilled water and then spread in a thin layer on dry filter paper and dried for 1 d at 30°C until the initial seed weight. These seeds were then packed in polythene bags having 12% moisture content and stored in refrigerator at 10°C for seven days [Afzal et al. 2011].

Seed germination capacity and seedling vigour evaluation. Four replicates of 25 treated or non-treated seeds were germinated in 9 cm diameter Petri dishes on Whatman No. 1 filter paper wetted with distilled water under continuous florescent light at 25°C

in a growth chamber (Vindon, England) for 7 days. Visible root protrusion was recognized as germination. The time to get 50% germination (T50) was calculated according to the formulae of Coolbear et al. [1980]. Mean germination time (MGT) was calculated according to Ellis and Roberts [1981]. Energy of germination and germination index (GI) were calculated as described in Handbook of Association of Official Seed Analysts [1983]. Final germination percentage (FGP) was calculated at the termination of experiment by dividing germinated seeds with total seeds and multiplying by 100.

For seedling vigour evaluation, control and treated seeds were sown in plastic trays (25 in each) containing moist sand, replicated 4 times and were placed in growth chamber (Vindon, England). Mean daily temperature in the growth chamber was 25°C with continuous florescent light during the course of investigation. Emergences were recorded daily on the basis of appearance of cotyledons on the surface by using Handbook of Association of Official Seed Analysts [1983]. Root and shoot lengths of emerged seedlings were determined at the end of experiment. All emerged seedlings were washed and separated into root and shoot for the determination of their fresh and dry weight. Dry weight was determined after oven drying the samples at 65°C for 3 days.

Biochemical analysis. α-amylase activity was measured in potassium phosphate (pH: 7.0) extracted samples of the seeds of French marigold (0.1 g), phenyl methyl sulfonyl fluoride (PMSF) (10 mM) was used as proteases inhibitor following the modified DNS method [Varavinit et al. 2002]. Total soluble sugars were quantified in (0.1 g) marigold sample after grinding with the help of mortar pestle followed by hydrolysis with 2.5N HCl and then neutralized by sodium carbonate. The distilled water was used to make final volume 10 ml, centrifuged at 10000 xg and supernatant was used for measurement of total sugars following the phenol-sulphuric acid method [Thimmaiah 2004]. The reducing sugars were measured by DNS method from the French marigold sample (0.1 g) extracted in 80% ethanol twice using 5 ml volume each time.

Statistical analysis. The experiment was repeated three times in a completely randomized design; data recorded each time were assembled for statistical analysis to determine the significance of variance ($P < 0.05$). For comparison of treatment means, standard errors were computed using Microsoft Excel programme.

RESULTS

Seed germination capacity and seedling vigour evaluation. Seed priming treatments significantly improved germination capacity and seedling vigour of the French marigold seeds as shown by reduced T50, MGT and increased GI, GE and FGP. Moreover, maximum final germination (from 57.3% to 81.3%), GI (from 12.2 to 23.95) and GE (from 32% to 61.3%) was recorded in haloprimed seeds ($CaCl_2$) as compared with non-treated and treated seeds (salicylic acid, ascorbate, hydropriming). Minimum T50 and MGT were noted in seeds subjected to $CaCl_2$ followed by salicylic acid (Tab. 1). Similarly, halopriming also resulted in maximum root and shoot lengths.

Seed priming treatments significantly improved the performance of the seeds by improving stand establishment attributes. Minimum MET and maximum EE, EI, FEP and improved root and shoot lengths were recorded by different priming treatments in fol-

Table 1. Effect of various French marigold seeds priming techniques on their germination capacity

Treatments	MGT (days)	T50 (days)	G.E. (%)	G.I	FGP (%)	Root length (cm)	Shoot length (cm)
Un-primed (Control)	5.96 a	3.72 a	32.00 c	12.24 c	57.33 b	2.50 c	1.50 c
Hydropriming	5.75 b	3.47 ab	34.67 bc	14.19 bc	57.33 b	3.30 b	2.00 b
50 mM $CaCl_2$	5.43 d	2.42 c	61.33 a	23.95 a	81.33 a	4.80 a	2.60 a
100 mgL^{-1} salicylic acid	5.63 c	2.87 bc	42.67 b	16.25 b	62.67 b	3.13 b	2.07 b
100 mgL^{-1} ascorbic acid	5.68 bc	3.17 ab	36.00 bc	15.36 bc	60 b	3.19 b	2.10 b
LSD	0.108	0.64	9.76	2.94	8.19	0.35	0.25

Figures not sharing the same letters in a column differ significantly at $p \leq 0.05$.
FGP = final germination percentage, GI = germination index, MGT = mean germination time,
T50 = time taken to 50% germination, GE = germination energy.

Table 2. Effect of various French marigold seeds priming techniques on seedling vigour

Treatments	MET (days)	E.E. (%)	E.I.	FEP (%)	Root length (cm)	Shoot length (cm)	Fresh weight ($mg\ seedling^{-1}$)	Dry weight ($mg\ seedling^{-1}$)
Un-primed (Control)	4.89 a	41.33 c	16.61 d	58.67 c	2.84 d	2.28 c	14.67 e	0.86 c
Hydropriming	4.78 b	57.33 b	20.86 cd	65.33 c	3.19 c	2.71 b	14.08 f	0.83 c
50 mM $CaCl_2$	4.49 d	80 a	36.18 a	88 a	5.14 a	3.38 a	38.93 a	2.29 a
100 mgL^{-1} salicylic acid	4.64 c	65.33 b	26.91 b	76 b	3.74 b	2.95 b	17.03 c	0.91 bc
100 mgL^{-1} ascorbic acid	4.72 b	61.33 b	24.64 bc	72 b	3.56 b	2.85 b	19.33 b	1.14 b
LSD	0.086	8.187	3.996	6.23	0.284	0.244	0.50	0.26

Means not sharing the same letters in a column differ significantly at $p \leq 0.05$.
FEP = final emergence percentage, E.I. = emergence index, MET = mean emergence time, E.E. = emergence energy.

lowing pattern $CaCl_2$ > SA > ascorbate > hydropriming > control (Tab. 2). Likewise, an increase in emergence capacity from 58.67% to 88% and decrease in MET from 4.89 days to 4.49 days was recorded in seeds primed with $CaCl_2$. In addition, seedling fresh and dry weight was also increased by priming with $CaCl_2$ followed by ascorbate and SA.

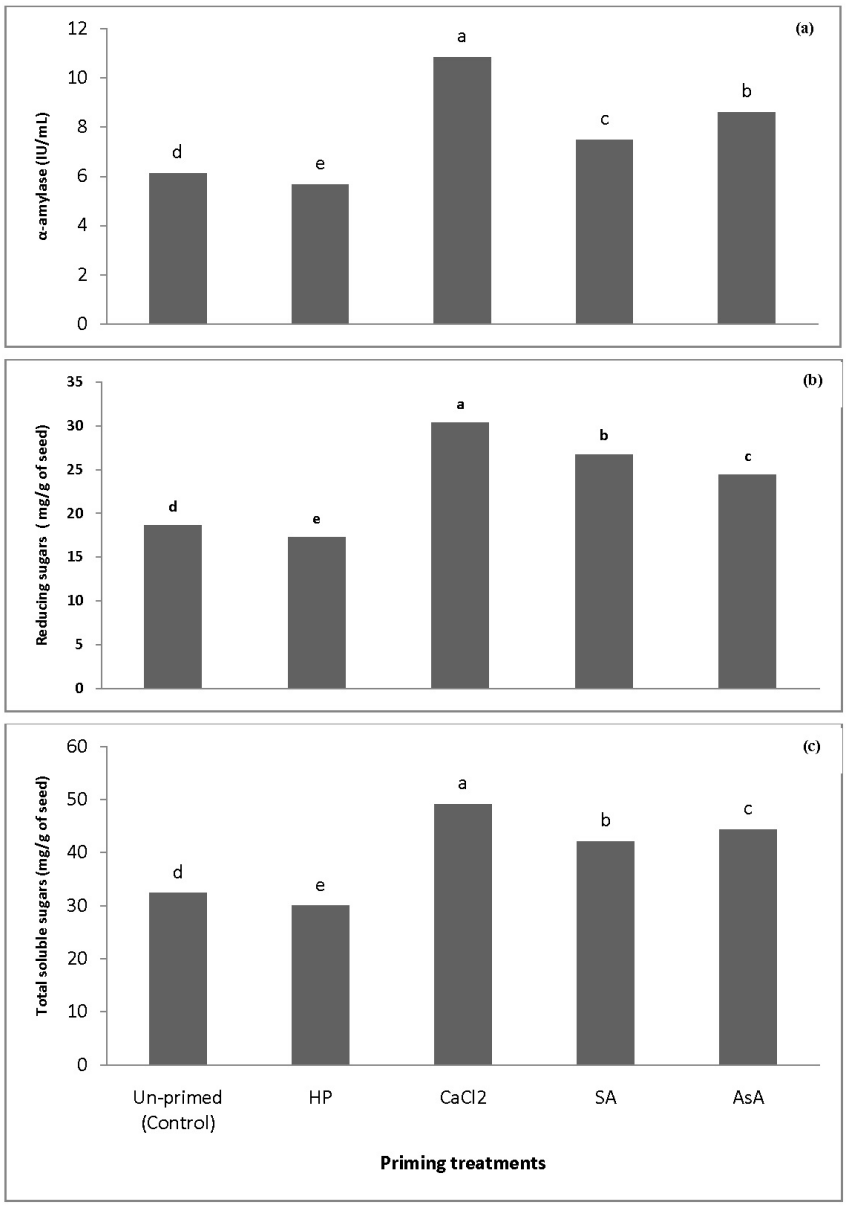

Fig. 1. Effect of different priming treatments on biochemical attributes of French marigold

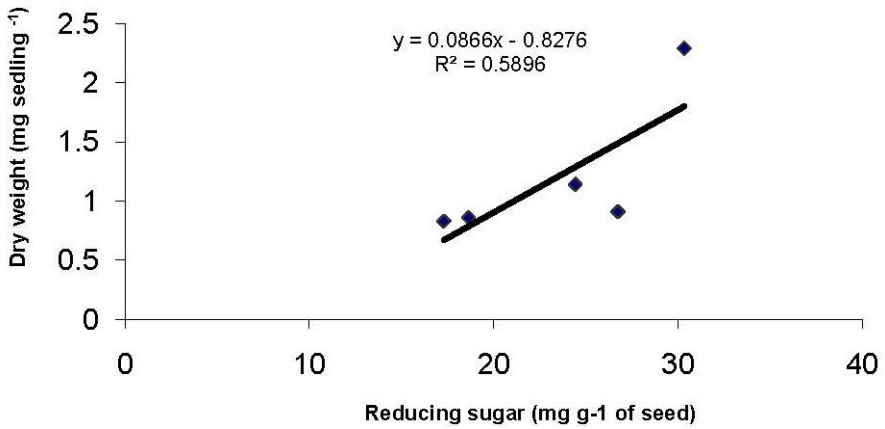

Fig. 2. Relationship between reducing sugars and seedling dry weight in the French marigold seeds as influenced by their priming treatments

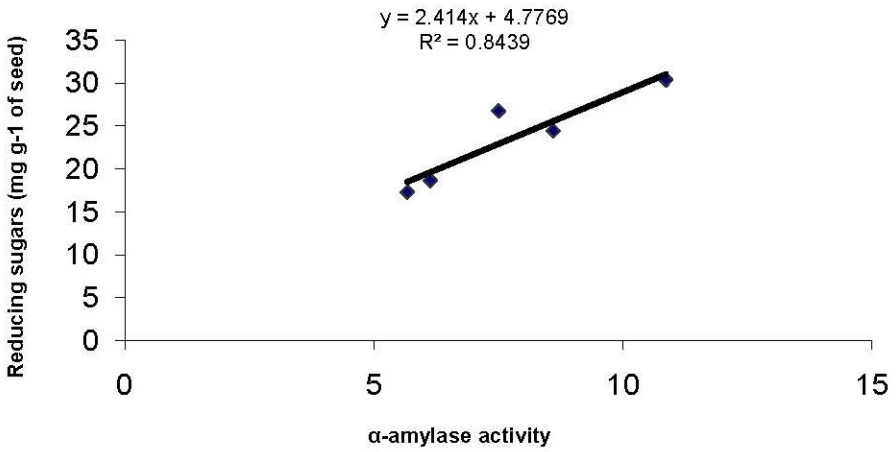

Fig. 3. Relationship between α-amylase activity and reducing sugars in the French marigold seeds as influenced by their priming treatments

α-amylase and sugar content analysis. A significant increase in α-amylase activity was recorded in all primed seeds of both cultivars but maximum response was recorded in seeds primed with $CaCl_2$ (Fig. 1a). All the priming agents significantly affected total and reducing sugars. Moreover reducing sugars and amylase activity were maximally increased in seeds by $CaCl_2$ followed by SA and ascorbate than hydropriming or non-primed seeds (Fig. 1b). Similar trend of seed priming treatments on total soluble sugars of French marigold seeds were recorded (Fig. 1c).

Correlation studies. There was a positive correlation between reducing sugars and dry weight (Fig. 2) and α-amylase activity and the concentration of reducing sugars (Fig. 3).

DISCUSSION

Seed enhancement is an easy, low cost, and low risk technique, and it is being used successfully to overwhelm the difficulties in seed germination and plant stand establishment [Afzal et al. 2011]. In present study, priming techniques considerably enhanced germination and seedling growth in the seeds of French marigold as compared to the non-primed seeds. But the effect of priming with 50 mM $CaCl_2$ was more pronounced in harmonizing germination as illustrated by lower T50, MGT and higher GI, GE, FGP. This improvement in germination and emergence performance of primed seeds might be due to repair mechanism, mobilization of storage reserves for utilization during germination and dormancy breakdown [Burgass and Powell 1984, Hocart et al. 1990]. The beneficial effect of priming has been associated with various biochemical, cellular and molecular events including synthesis of DNA and proteins [Bray 1995]. Metabolic repair processes and buildup of germination metabolites or osmotic adjustments during priming are due of germination enhancement techniques [Bradford 1986].

Shoot and root length was increased due to the application of priming with $CaCl_2$ followed by SA and ascorbate and this increased shoot and root length as compared to control might be increased rate of cell division in the root tips and earlier start of emergence as indicated by lower values of MET and E50 [Afzal et al. 2011]. Higher cell wall extensibility and higher metabolic processes resulted in increase in root shoot ratio [Haghpanah et al. 2009]. Priming with calcium chloride made the nutrients available to the plants, hence, causing extensibility in cell wall of the roots and an increase in seed respiration intensity [Orzesko and Podlaski 2003]. Non-primed seeds resulted in less seedling vigour in terms of root shoot length and dry of weight of seedling which was due to decreased emergence and higher values of E50. The positive correlation between reducing sugars and seedling dry weight suggest that seed priming treatments remarkably improved seedling vigour due to increased starch hydrolysis.

An increased α-amylase activity along with contents of total and reducing sugars of primed seeds was observed in this present study (Fig. 1). It confirms the important role of priming in either inducing the *de novo* synthesis or increasing the activities of hydrolytic enzymes thereby producing germination metabolites in requisite amounts [Sung and Chang 1993, Lee and Kim 2000]. Positive correlation between α-amylase and reducing sugars also confirms the assumption that increased α-amylase resulted in increased reducing sugars.

On the basis of results obtained from this investigation, it is concluded that priming with $CaCl_2$ followed by salicylic acid and ascorbate showed maximum seed invigoration and better performance in French marigold seeds. The findings of this study is important in understanding the direct physiological effect of priming in enhancing seed vigour and stand establishment in marigold and must give precise and reliable information to the seed industry regarding potential effectiveness of priming on marigolds.

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CZY KONDYCJONOWANIE WZMAGA KIEŁKOWANIE NASION I WSCHODY AKSAMITKI (*Tagetes patula* L.) POPRZEZ WYWOŁANIE ZMIAN FIZJOLOGICZNYCH I BIOCHEMICZNYCH?

Streszczenie. Słabe wschody to powszechny problem w produkcji aksamitki. Kondycjonowanie jest jedną ze strategii stosowanych w celu przyspieszenia wzrostu roślin, zwłaszcza kiełkowania nasion. Przeprowadzono więc eksperyment mający na celu zbadanie wpływu różnych technik kondycjonowania na rozwój aksamitki. Oceniono różne techniki kondycjonowania nasion: hydrokondycjonowanie, 50 mM $CaCl_2$, 100 mg L^{-1} kwasu salicylowego oraz 100 mg L^{-1} kwasu askorbinowego. Kontrolę stanowiły nasiona bez żadnych zabiegów. Okazało się, że kondycjonowanie za pomocą 50 mM $CaCl_2$ było najbardziej skutecznym zabiegiem wzmagającym kiełkowanie i żywotność siewek w porównaniu ze wszystkimi innymi zabiegami, łącznie z kontrolą, co było widoczne na podstawie wyższego końcowego kiełkowania/wschodów, energii kiełkowania oraz mniejszego czasu kiełkowania/wschodów. Ponadto, dodatnia korelacja między cukrami redukującymi a suchą masą i aktywnością α-amylazy a także stężenie cukrów redukujących wskazujące na wzmożony wzrost będący efektem kondycjonowania można przypisywać wyższej zawartości cukrów redukujących i całkowitej zawartości cukrów oraz wyższej aktywności α-amylazy w nasionach poddanych kondycjonowaniu.

Słowa kluczowe: kiełkowanie, kondycjonowanie, żywotność siewek, parametry jakości nasion

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