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Optimizing pre-sowing seed treatments for accelerating synchronized germination, better crop establishment, nodulation, low incidence of wilt and *Ascochyta* blight, and high yield in chickpea under sodic soil

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ABSTRACT

Chickpea (*Cicer arietinum*), being a globally important pulse crop, is contributing about 45 per cent share in total pulse production and 71 per cent in total pulse export of India. Global chickpea market will register a cumulative annual growth rate (CAGR) of almost 4 per cent by 2022. Thus, concerted efforts should be made in order to mitigate the production constraints especially in delaying the seed germination, poor plant stand and wilting in sodic soil condition. Uniform, healthy and dry seed (9% moisture) of chickpea cv. Pusa 362 were, therefore, subjected to pre-sowing seed treatments *viz.,* hydro (soaking in water), GA_3 (50 ppm), KNO_3 (0.2%), KCL (2%), *Trichoderma viride* (5g/ kg seed), *Rhizobium* inoculation (20g/kg seed) and thiram (3g/kg seed) for 8 hrs in order to investigate their suitability for coping up of the main constraints *viz.,* poor germination, crop establishment, nodulation, comparatively high incidence of wilt and *Ascochyta* blight, and finally low yield under sodic soil condition (*p*H above 8.5). Hydroprimed seed (8 hrs) dressed by either thiram (3g/kg seed) or *Trichoderma viride* (5g/kg seed) and inoculated with *Rhizobium* (20 g/kg seed) followed by seed primed with either GA_3 (50ppm) or KNO_3 (0.20%) for 8 hrs accompanied by its dressing with either thiram (3g/kg seed) or *Trichoderma viride* (5g/kg seed) and *Rhizobium* inoculation (20 g/kg seed) need to be exploited for acceleration and synchronized germination, better crop establishment, increased nodulation and seed yield of chickpea in particular and in general of other pulse crops under sodic soil condition.

Key words: Acceleration and synchronized germination, Chickpea, Nodulation, Seed priming, Seed yield

Pulses, being a rich source of protein (20-25%) and fixing ability of atmospheric nitrogen to N-compounds in soil (72-350 kg/ha), are vital constituent of healthy diet and cropping, respectively. India contributes about 35 per cent share in global area and production and is thus, largest producer and consumer of pulses. During 2017-18, pulses were grown in an acreage of over more than 29 million ha and recorded the highest ever production of 25.23 million tonnes with a productivity level of 841 kg/ha. The exponential growth rate in production of pulses during last year was recorded as more than 9 per cent. Thus, India is pride to achieve the ever highest production of 25.23 million tonnes of pulses during 2017-18 making ahead the nation self sufficient in pulses. Further, during 2017-18, an approximate increase in pulses has been to the tune of 19 per cent area and 34 per cent production and 13 per cent yield, which consequently led to increase its per capita availability. As such the country is seemed now quite confident of meeting the projected demand of 35 million tonnes by 2030. However, uncertainty in growth is still reckoned due to the vagaries of the weather and the fact that out of total 141.40 million hectares net sown area, 73.20 million hectares (52%) are still un-irrigated and rain fed. More than 80 per cent of total pulses are grown in such region. Besides, salt affected soil occupies about 6.74 million hectares area of which 56 per cent are sodic and remainder 44 per cent saline (Sharma *et al*. 2016). Sodic soils contain a large number of exchangeable sodium and low levels of soluble salts. Keeping these all together, Dheer *et al.* (2018) estimated production of pulses would be around 35 MT by 2050. Among pulses, chickpea is the leading one which recorded a highest ever production of 11.23 million tonnes at a record productivity level of 1063 kg/ha in an area of 10.56 million hectares. It is the single largest share in India's basket of pulses registering 70.92 per cent share in the total pulses export (179.36 thousand) and 17.50 per cent share in total pulses import (5607.53 thousand tonnes) during 2017-18 (Commodity profile for pulses-July, 2018, Department of Agriculture Cooperation). Tecnavio,s analysis have predicted that the global chickpea market will register a CAGR of almost 4 per cent by 2022 (Global chickpea market 2018-2022, www.reportbuyer.com). Chickpea bearing comparatively high number of branches and a large sized seed, matures in often indeterminate mode. Thus, seed ageing and imbibitions damage and their interaction concert the approaches to seed enhancement in grain legume particularly chickpea (Legesse, 1991; Powell, 1998). Further, it is very salt-sensitive pulse crop and that's why salts have more adverse effects on germination, plant establishment, nodulation, N_2-fixation, growth, flowering, pod formation and retention and seed filling and finally seed yield (Flowers *et al*. 2010 ; Kamdambot *et al.* 2010). Seed priming (pre-sowing treatments) is now being realized for rapid and uniform germination, better crop stand and increased yield of course, as per genetic potential of crops/

varieties (Heydecker 1973; Gray and Steckel 1977; Parera and Cantiliffe 2010; Wilkinson 1918; Yadav 2018). Alleviation of salt stress of black glutinous rice by seed priming with spermidine and gibberellic acid (Chunthaburee *et al*. 2014), faba bean by Trichoderma sp. (Abd El-Baki and Mustafa 2014), in maize with chloride salts (Asraf and Rauf 2001) and with seed priming in soybean (Basra *et al.* 2005) and in wheat (Basra *et al.* 2006) have been reported. However, very limited studies have been taken up on seed priming on chickpea (Elkoca *et al.* 2007). Keeping these in view, the present investigation was subjected to six diverse priming treatments namely hydro, GA_3 (50 ppm), KNO_3 (0.2%), KCL (2%), *Trichoderma viride* (5g/kg seed), *Rhizobium* inoculation (20g/kg seed) and thiram (3g/kg seed) to explore their suitability for rapid and uniform germination, better crop stand, nodulation and increased yield in chickpea.

MATERIALS AND METHODS

Uniform and healthy seed (moisture 9%) of chickpea cv. Pusa 362 were surface sterilized after immersing completely in 0.10% mercuric chloride for about 2-3 minutes followed by washing with distilled water. These surface sterilized seed were subjected to priming treatments namely hydro (soaking in water), GA_3 (50 ppm), KNO_3 (0.2%) and KCL (2%) for 8 hrs separately following v/w, thoroughly washed 3-4 times to remove chemicals/residues from seed surface and followed by their drying under ambient condition till the seed resumed in its original moisture.

Further, apart from hydro priming treatment, hydro primed seed were used for seed dressing with Thiram (3g/kg seed), *Trichoderma viride* (5g/kg seed), *Rhizobium* inoculation (20g/kg seed) separately. Dry un-primed seed were treated as control. One hundred seed of each treatment were kept in rolled towels in three replications and placed in BOD incubator at 25+-1^{0}C and daily observations were recorded.

Beside laboratory experiments, two sets of field experiment were also conducted. In the first set, 100 seed of each treatment were tested in field with four replications. The number of normal plants was counted at the end of 14 days and the field emergence percentage was calculated. In the second set, the experiment was conducted accommodating all treatments in randomized block design with three replications in plot size of 5 m × 4 m. The spacing between rows was kept 40 cm apart and the plant to plant was 10 cm. Recommended package of practices were adopted time to time in order to raise an ideal crop. Ten random plants were scooped out from each replication at flowering stage, carefully washed their root system and counted nodules their on. Nodules were further subjected for their fresh and dry weight. Besides, ten other plants were randomly selected replication wise to record plant height, number of pods and seed per plant. Incidence of wilt and *Ascochyta* blight were also recorded on plant basis where as seed yield on plot basis. The crop was harvested when plants started drying, the colour of leaflets turned pale and pods to yellow colour. The harvested plants were dried in the shade. The seed were separated manually by gently beating the dried plants with the wooden stick. The seed were cleaned and dried in the shade until seed moisture content reduced upto 8-9 per cent. One thousand seed in 4 replications were counted manually from seed lot in each treatment and their weight was recorded by adopting ISTA procedure (Anonymous 1999) and expressed in grams. For germination,100 seed were used by adopting the between paper towel method, kept at optimum condition of temperature (25^{0}C+-1^{0}C) and relative humidity (95+-1% RH) in four replications (Anonymous 1999). The number of normal seedlings was counted at the end of 8 days (Final count) and the germination percentage was calculated. Further, for observation related to speed of germination, 100 seed in four replications were placed in rolled to well and incubated at 25+-1. Daily count on germinated seed was made up to 8th day. The speed of germination and seedling vigour index were calculated according to Haydecker (1969) and Abdul-Baki and Anderson (1973), respectively. Data were subjected to statistical analysis following standard methods (Panse and Sukhatme 1967).

RESULTS AND DISCUSSION

All the pre sowing treatments significantly increased the germination, speed of germination, root length, shoot length and seedling vigour index in comparison to control (Table 1). The maximum seed germination (90.51%) was recorded with Thiram followed by hydro priming (90.25%). Seed primed with KNO_3 (0.2%) influenced maximum speed of germination (51.45) which very closely (51.40) followed by GA_3 (50 ppm). Further, GA_3 (50 ppm) increased maximum root length (16.20 cm) and shoot length (5.06 cm) followed by *Trichoderma viridi*. The maximum seedling vigour index (1900) was recorded by seed primed with GA_3 (50 ppm) followed by *Trichoderma viride* (1782). The field emergence and crop establishment both were almost equal and maximum with seed primed with *Trichoderma viride* (5g/kg seed)/ Thiram (3g/kg seed). Further, these both field emergence and crop establishment were significantly increased by rest pre-soaking treatments in comparison to control (Table 1). Thus, it is clearly appeared that there was a differential response of treatments with germination behaviour and plant stand which might be due to the different mode of action of these treatments though the ultimate effect was to enhance the synchronized germination, seedling growth and optimum plant stand. Almost similar observations of seed priming with hydro (Sen and Osborne 1974), GA_3 (Brockkhurst *et al.* 1982; Yamada *et al.* 1962), KNO_3 (Heydecker *et al.* 1973; Shinde and Jadav 1995; Wiebe and Muhyaddin 1987) and with bioagents (Harman and Tylor 1988; Upadhayay *et al*. 1993) have been recorded. Further, Farhoudi and Tafti (2012)

Table 1. Effect of seed priming on germination behaviour and crop establishment in chickpea Pusa 362

Treatment	Germination (%)	Germination Speed	Root length (cm)	Shoot length (cm)	Vigour index	Field emergence (%)	Crop establishment (%)
Hydropriming (8 hrs)	90.25	50.05	14.50	4.65	1728	87.50	80.34
GA_3 (50 ppm)	89.41	51.40	16.20	5.06	1900	87.56	81.56
KNO_3 (0.2%)	88.07	51.45	14.36	4.70	1679	86.30	80.93
KCl (2%)	86.89	50.21	14.52	4.60	1661	85.04	79.94
Trichoderma viride (5g/kg seed)	89.63	51.38	15.09	4.79	1782	88.37	81.86
Rhizobium (20g/kg seed)	89.93	49.85	14.08	4.86	1703	85.50	80.37
Thiram (3g/kg seed)	90.51	50.54	14.91	4.60	1766	88.00	81.81
Control	85.33	44.25	13.53	4.25	1518	82.00	78.00
C.D. (5%)	3.45	4.76	0.45	0.52	127	2.36	1.58

reported that the seed priming with 1 and 2 per cent solution of KNO_3 significantly improved germination, seedling growth, and peroxidase activity, and decreased lipid peroxidation under salt stress. A differential response to seed priming among seed lots of same species has also been reported by Haigh and Barlow (1987) and Bradford *et al.* (1990). High vigour and seed free from pathogens are both essential prerequisites for good priming results (Cantliffe *et al.* 1987).

Effects of pre-soaking treatments on nodulation in chickpea cv. Pusa 362 are presented in Table 2. The maximum number of nodules and their fresh and dry weights/plant were observed by the seed inoculation with *Rhizobium*. Other treatments increased these attributes of nodulation but non-significant in nature as compared to control. The benefit of *Rhizobium* inoculation on nodulation, nitrogen fixatition, dry matter accumulatuion, seed yield and seed quality of legumes in soil devoid of any Rhizobial strain is well established (Bushby *et al.* 1983; Yadav *et al.* 2014). Gupta *et al.* (2004) reported that Rhizobium inoculated olants showed increased plant height growth at 30 and 60 DAS, growth of root and shoot, biomass, chlorophyll and carotenoids in chickpea vars SG 8962 and C 235. Kumpawat and Manohar (1994) reported, Rhizobium inoculation significantly increased the number and dry weight of nodules per plant, protein content and increased seed yield.

The seed yield and its important contributing traits *viz.*, number of branches/plant, number of pods/plant and number of seed/plant were significantly increased in all seed priming treatments as compared to the control (Table 3). The maximum being with the Rhizobium inoculation followed by seed treatment done with GA_3 (50 ppm). Besides, rest treatments were significantly increased seed yield and its contributing traits except to test weight as compared to the control.

Table 2. Effect of seed priming on nodulation in chickpea Pusa 362

Treatment	Number of nodule/ plant	Fresh weight of nodule/ plant (g)	Dry weight of nodule/ plant (mg)
Hydropriming	10.54	0.12	0.035
GA_3 (50 ppm)	11.20	0.11	0.037
KNO_3 (0.2%)	10.64	0.12	0.036
KCl (2%)	10.50	0.12	0.037
Trichoderma viride (5g/kg seed)	11.14	0.13	0.038
Rhizobium (20g/kg seed)	20.58	0.22	0.106
Thiram (3g/kg seed)	11.32	0.11	0.032
Control	09.24	0.09	0.016
C.D. (p=0.05)	4.68	0.06	0.023

The growth regulators are known to exert significant influence on plant structure, crop growth, seed yield and quality attributes in different pulses. In the present study also growth regulators have shown the marked stimulatory effect on various traits. The increase in root and shoot length is attributed to their influence on increased cell division, elongation and growth promoting nature of the chemicals (Setia *et al.* 1991).

Increased branches noticed might be due to the increased supply of nutrients in *Rhizobium* inoculation thereby enhanced growth (Jain *et al.* 2014). The Rhizobium helps more fixation of atmospheric nitrogen in nodules as aspergine and glutathiamin which increase the production of protein, carbohydrates and starch resulting in greater dry matter accumulation and branches per plant (Reddy and Reddi 1992). The marked differences were noticed among different seed treatments for seed yield and yield parameters. Higher seed yield was obtained in *Rhizobium* seed treatment followed by GA_3 as compared to other treatments and control. The increase in seed yield in these treatment may be due to more nitrogen availability through increased nitrogen fixation, high number of pods/plant,

Table 3. Effect of seed priming on seed yield and its important contributing traits in chickpea Pusa 362

Treatment	Branches/ plant	Pod/ plant	Seed/ plant	1000 seed weight (g)	Seed yield (kg/ha)
Hydropriming	21.51	46.41	88.16	250	2223
GA_3 (50 ppm)	22.23	51.28	98.57	250	2358
KNO_3 (0.2%)	22.05	49.50	95.71	251	2284
KCl (2%)	21.87	49.27	93.85	252	2267
Trichoderma viride (5g/kg seed)	21.74	48.68	92.12	251	2259
Rhizobium (20g/kg seed)	23.16	50.72	95.54	253	2375
Thiram (3g/kg seed)	21.83	48.21	90.24	252	2214
Control	17.21	34.65	65.53	248	1823
C.D. (p=0.05)	3.12	10.28	18.67	NS	176

seed per pod and greater yield attributes via higher photosynthetic rate and their translocation to sink contribute for higher seed yield (Jat and Ahlawat 2004). Further, inoculation of *Rhizobium* is known to produce the endogenous growth regulators and release ammonia from *Rhizobium* resulting in higher yield (Srinivas and Shaik 2002). Besides, seed quality attributes such as 1000 seed weight, germination, seedling length, seedling vigour index, speed of germination were found quite considerable with *Rhizobium* (20g/kg seed) as compared to control and other treatments. Generally, the seed quality is associated with higher seed size, weight and food reserves. Germination and seedling growth depend upon the initial supply of food reserves.

Thiram is the simplest thiuram disulfide and the oxidized dimer of dimethyldithio carbamate. It is used as a fungicide, ectoparasiticide to prevent fungal diseases in seed and crops. *Trichoderma viride* is a fungus and a biofungicide. It is used for seed and soil treatment for suppression of various diseases caused by fungal pathogens. *Fusarium wilt*, caused by the soil borne fungus *Fusarium oxysporum* f,sp. *Ciceris*, has become a major factor limiting chickpea production worldwide. Development and use of high-yielding cultivars resistant to the prevelent pathogen race(s) in a given area is the most ptarcical and cost-efficient individual disease control measure for management of the disease. Besides, use of certified seed free from diseases sanitation and cropping practices to reduce inococulum in soiland protection of healthy seed with fungicides or biocontrol agents, would be of help for the management of *Fusarium* wilt in chickpea. (Meki *et al.* 2011; Rafel and Juan 2015). *Ascochyta* blight is the most frequent and damaging disease of chickpea worldwide. It is caused by *Ascochyta rabiei* [(Pass.)Labr.], a fungus that selectively attacks chickpea. Then persists in the crop,s residues, seed and volunteer plants. Infections may arise from seed borne inoculums or from wind borne spores *i e*. ascospores (Wiese *et al*. 1914: Nene *et al*. 2012). Seed primed with Thiram (3g/kg seed) showed significantly lower incidence of wilt (0.93%) and *Ascochyta* blight index (2.12) followed by *Trichoderma viride* (5g/kg seed) in comparison to 7.33 per cent and 18.23, respectively in the control (Table 4). Thus, seed hydroprimed for 8 hrs followed by dressing with Thiram (3g/kg seed) and Trichoderma viridi (5g/kg seed) will highly be effective in management of wilt and *Ascochyta* blight in chickpea. Salt stress owing to excessive amount of soluble salts in the root zone, induce osmotic stress and ion toxicity in the growing plant. Among toxic ions, sodium (Na^+) has the most adverse effects on plant growth by its detrimental influence on plant metabolism in inhibiting enzyme activities. Optimal potassium (K^+): Na^+ ratio is vital to activate enzymatic reactions in the cytoplasm necessary for the maintenance of plant growth and yield development. Although most soils have adequate amount of K^+, in many soils available K^+ has become insufficient because of large amount of K^+ has been utilized by high yielding crops. This problem is exacerbated under sodic or saline-sodic soil conditions as a consequence of K^+-Na^+ antagonism. Thus, K^+ uptake by plants is severely affected by the presence of Na^+ in the nutrient medium. Minimizing Na^+ uptake and preventing K^+ losses from the cell may help to maintain a K^+: Na^+ ratio optimum for plant metabolism in the cytoplasm under salt stress conditions (Wakeel 2013). Seed priming with KNO_3 may played to maintain an optimum ratio between K^+ and Na^+. The present findings on this aspect are in accordance to the earlier workers (Afzal *et al*. 2007; Ashraf and Foolad 2005).

Table 4. Effect of priming treatments on wilt incidence and *Ascochyta* blight of chickpea cv. Pusa 362

Treatment	Wilt incidence (%)	*Aschochyta* blight (PDI)
Hydropriming	6.02	13.87
GA_3 (50 ppm)	6.84	11.62
KNO_3 (0.2%)	5.69	12.41
KCl (2%)	6.76	13.25
Trichoderma viride (5g/kg seed)	2.43	4.59
Rhizobium (20g/kg seed)	5.28	12.56
Thiram (3g/kg seed)	1.93	3.12
Control	7.33	18.23
C.D. (5%)	2.54	7.86

It could therefore, be inferred from the above that hydroprimed seed (8 hrs) dressed by either thiram (3g/kg seed) or *Trichoderma viride* (5g/kg seed) and inoculated with *Rhizobium* (20 g/kg seed) followed by seed primed with either GA_3 (50ppm) or KNO_3 (0.20%) for 8 hrs accompanied by its dressing with either Thiram (3g/kg seed) or *Trichoderma viride* (5g/kg seed) and *Rhizobium* inoculation (20 g/kg seed) need to be exploited for acceleration and synchronized germination, better crop establishment, increased nodulation and seed yield of chickpea in particular and in general of other pulse crops under sodic soil condition.

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