Enhancing Germination and Seedling Vigour of Interspecific F_1 Hybrid Chilli Seeds through Priming with KNO_3 and GA_3

Abstract

India is called a home for spices and also involved in the export. Chilli is an indispensable spice, essentially used in every Indian cuisine due to its pungency, taste, appealing odour and flavour. Quality seed is a key component for the successful agriculture, where the main objective is each seed should germinate and produce a vigorous seedling which ensures higher seed yield, productivity and also better storability to achieve good yield in the coming season. To achieve all the above said traits, the seed technologists have developed seed enhancement techniques. Seed enhancement techniques are post-harvest treatment of seeds to enhance the seed germination and seedling growth by facilitating the delivery of seeds with other materials at sowing time. This enhancement technique includes hydration treatments like seed priming and seed encapsulation like seed pelleting, which impacts significantly on seedling emergence and establishment. In this experiment study was conducted different priming treatments, seeds primed with KNO_3 @ 1%, 2% and 3% and seeds primed with GA_3 @ 50 ppm, 100 ppm and 150 ppm, the seeds treated with KNO_3 @ 1% showed highest germination percentage, root, shoot, seedling length, seedling dry weight and seedling vigour index I and II which was followed by GA_3 @ 50 ppm. Acknowledge of proper priming techniques in order to improve the germination in the seeds.

Key words : KNO_3, GA_3, and seed priming.

Introduction

Capsicum is, at least economic-wise, one of the essential spices cum vegetable crop belonging to Solanaceae family with diploid chromosome number 2n=24, which has about 90 genera, and 2000 species. India is the primary producer, consumer and exporter of chilli globally. This crop is mainly cultivated in tropical and subtropical countries *viz*., India, Africa, Japan, Mexico, Turkey and USA etc. Chilli originates from South and Central America [1]. Chilli pepper fruits constitute large amounts of beneficial compounds including carbohydrates, minerals, proteins, amino acids, antioxidants, phytochemicals, and vitamins [2]. Capsicum fruits could be employed in food and medicine as a source of natural antibacterial agents. The nutritional value, flavour, aroma, texture, and colour of several chilli components are all essential. Chilli is valued for two qualities: its red colour, which comes from the pigment capsanthin and its piercing pungency, which comes from capsaicin.

Average yield of chilli in North Karnataka is higher than in South Karnataka, due to the difference in the soil and climatic conditions and lack of resistant varieties especially suited to its soil and climatic conditions of South Karnataka [3] .During 2020-21, Indian chilli occupied an area of 6.83 lakh hectares (16.87 lakh acres) with a production of 17.02 lakh tonnes and productivity of 2.494 tonnes per hectare (1.009

tonnes per acre). In India, major chilli producing states are Andhra Pradesh (6.60 lakh tonnes), Telangana (3.28 lakh tonnes), Madhya Pradesh (2.18 lakh tonnes), Karnataka (1.80 lakh tonnes) and West Bengal (1.04 lakh tonnes) accounting for 6 per cent of all India production respectively [4].

Even though India ranks first in chilli area and production, the yield potential is low due to poor yielding varieties and high incidence of pests and diseases. One of the methods to achieve quantum jump in yield and quality is heterosis breeding. Hybridization between pepper varieties or species was generally used for fundamental research to identify genes of interest, but more recently it has become common place as a breeding tool per se [5]. Hybridization breeding allows the combination of dominantly inherited traits, including disease resistance and agronomic traits [6].

The interspecific crosses are incompatible and if compatible the getting viable seeds are negligible. However, the germination of interspecific viable seeds is very slow and if germinated, growth of the seedlings is either stunting or no further survival. Hence, there is a need to improve and fasten the seed germination of interspecific hybrid seeds in chilli.

It has been reported that chilli seed germination is slow and non-uniform under normal as well as abiotic stress conditions including moisture and cold stresses [7].Priming treatments give a head start to the speed of germination and uniformity of growth by fixation of metabolic activities over un-primed seeds and also synchronization of metabolism of all the seeds in a seed lot [8]. Numerous priming techniques have been found to be valuable pre-sowing seed treatments that upsurge the speed of germination and seedling development, as well as advance the pre-metabolic activities to withstand the field stress conditions such as deficiency of water or hostile temperatures.

Hydropriming introduces liquid water to seeds in controlled and precise amounts to achieve a desired level of hydration [9]. It allows the seeds to quickly reach a high level of moisture with a constant supply of oxygen, thus increasing the level of metabolites associated with the germination process (intermediate metabolites) and enzymes associated with the production of energy [10].

Soaking or treating of seeds in optimal concentrations of plant growth regulators (PGRs) to improve germination, stand establishment, plant growth and yield of crop plants under both normal and stress conditions is referred to as hormonal priming [11]. Several growth regulators are commonly used for seed priming including auxins, gibberellins, cytokinins, abscisic acids, polyamines, brassinolide, salicylic acid, triacontanol and ascorbic acid. The main objective of this study is to obtain the best pre soaking seed treatment for better germination of distant hybrid seeds.

Materials and Methods

To improve the germination of distant hybrid seed through *in vitro* techniques, we followed some pre sowing treatments and the procedure followed for treatments is seeds were surface sterilized by dipping the seeds in sodium hypochlorite (5 %) solution for 5 minutes and dried on filter paper. Priming solutions are prepared according to the dosage specific for the treatment. The duration of priming varies with the priming material used for the treatment. Here the treatments KNO_3 (1%), KNO_3 (2%) and KNO_3 (3%) is primed for 12 hrs; GA_3 (100 ppm), GA_3 (150 ppm) for 24 hrs.

During priming, the seeds were kept in dark condition @ 25± 2°C. After respective priming treatments for specific period, seeds were washed with distilled water thoroughly. Then primed seeds were dried at room temperature on filter paper for 24 hours, later to their original moisture contents. Dried seeds were then packed in polythene bags and stored for further use.For germination test three hundred seeds were randomly taken from each treatment of chilli. Three replicates of 100 seeds were germinated between double layered rolled germination paper along with control (dry seeds) and moistened with sterile distilled water, in an amount equivalent to 2.5 times the mass of dry substrate, made into rolls, and placed into a seed germinator at 25ºC. Germination was considered to have occurred when the radicles were 3 mm long. The seedlings with short, thick and spiral form hypocotyls and stunted roots were considered as abnormally germinated. Germination percentage was recorded on fourteenth day (final count). Following are the few seed quality parameters are recorded in the course of present investigation.

Seed germination (%)

The germination test was conducted in the laboratory using between paper method as per ISTA rules [12]. A total of 400 seeds were randomly selected from each treatment and grouped into four replications of 100 seeds each. Seeds were placed equidistantly on moist germination paper. The paper towels were rolled along with polythene cover and banded with rubber band. The rolled towels were incubated in germination chamber maintained at 25 ± 1 ºC temperature and 90 ± 2 per cent relative humidity (RH). The first count and final count of normal germinated seedlings were taken on 7^{th} and 14^{th} day after germination, respectively. The percentage of germination was expressed based on normal seedlings present in the test.

$$\text{Seed germination (\%)} = \frac{\text{Number of seed germinated}}{\text{Total number of seeds put for germination}} \times 100$$

Mean seedling length (cm)

Ten normal seedlings were randomly selected from each treatment and replicationwhich are put for germination test and then carefully separated from the paper towel of thegermination test. The total length of the seedling was measured from tip of primary root totip of primary leaf by using a measuring scale. The mean of ten seedlings

from eachtreatment in each replication was calculated. Then it is expressed in centimetres [12].

Mean seedling dry weight (mg)

Ten normal seedlings were selected randomly from the germinated seedlings formeasuring the seedling length, the same were placed in the butter paper bag by removingthe cotyledon and dried in a hot air oven, maintained at 83 ± 1˚C temperature for 17 hours. Then the seedlings which were removed are allowed to cool in a desiccator for 30 minutes.The dried seedlings were weighed in an electric balance and the weight was recorded. Thenit is expressed in milligram [13].

Seedling vigour index –I

From the seeds of germination test by employing between paper method, the seedlings that were germinated are evaluated on 7^{th} and 14^{th} day as first and final count respectively and the percentage of germination was expressed based on the normal seedlings present in the test. Then ten normal seedlings were selected randomly on 14^{th} day and mean seedling length (shoot and root) was measured in centimetres.

The seedling vigour index - I was calculated as per the formula suggested by [14], by multiplying standard germination percentage with mean seedling length (cm) and expressed in whole number for each treatment.

SVI - I = Seed germination (%) × Mean seedling length (cm)

Seedling vigour index –II

From the seeds of germination test by employing between paper method, the seedlings that were germinated are evaluated on 7^{th} and 14^{th} day as first and final count respectively and the percentage of germination was expressed based on the normal seedlings present in the test. Then ten normal seedlings were selected randomly on 14^{th} day and mean seedling length (shoot and root) was measured in centimetres. Then those seedlings were dried in hot air oven maintained at 83 ± 1°C for 17 hours and cooled in the desiccator for 30 minutes. The mean seedling dry weight was recorded and expressed in milligrams.

Seedling vigour index-II was recorded by multiplying the standard germination percentage and seedling dry weight. It was computed by adopting the formula as suggested by [14] and expressed in whole number.

SVI-II = Seed germination (%) × Mean seedling dry weight (mg)

The experimental data collected on various seed quality parameters were subjected to the analysis of variance by adopting the appropriate methods as outlined by [15] The level of significance used in ‘F’ test was at P = 0.01. Whenever F-test was significant for

comparison amongst the treatments an appropriate value of critical difference (CD) was worked out.

Result and Discussion

The primary goal of this study was to increase the germination of distant hybrid seed because interspecific F_1 hybrid seeds of chilli have poor germination. There are various ways for improving germination, one of which is seed dormancy breaking by using seed dormancy breaking chemicals, which has been used in this study. Seed priming is the most significant and effective method for promoting germination. As a result, chemicals such as KNO_3 and GA_3, were utilized at different concentrations in this study. Among the 8 different priming treatments, the best three treatments were identified based on seed quality testing for which the statistically analysed data of germination percentage, mean seedling length (cm), mean root length (cm), mean shoot length (cm), mean seedling dry weight (g), seedling vigour index-I and IIare presented in Table **1, 2 and 3**.

Seed germination (%)

The data on seed germination (%) of UARCH-42, PBC-80 and F_1interspecific hybrid as influenced by seed primed are presented in Table 1, 2 and 3and also represented in Fig. 1, 2 and 3. The seed germination differed significantly among the seed treatments and it ranged from 88.00 to76.00 per cent, 79.04 to 69.04 per cent and 9.00 to 0.00 per cent, for UARChH-42, PBC-80 and interspecific hybrid respectfully. The highest seed germination of 88.00, 79.04 and 9.00 per cent was recorded when seeds were primed with treatment @ 1 per centconcentration KNO_3, which was on par with 86.00, 77.04 and 0.00 per cent germination in KNO_3 @ 2per cent, followed by 84.00, 75.04and 0.00 per cent germination in GA_3 @ 50 ppm. Whereas, the control recorded the lowest seed germination of 76.00, 69.04 and 0.00 per cent which was significantly lower than other treatments and followed by seeds primed with distilled water for 24 hrs (78.00, 70.00 and 0.00 per cent).

The increase in germination of the invigorated seeds is due to repair of cell organelles, enzymatic reactions, activation of metabolic process of food reserves and rejuvenation of embryo viability [16]. The decrease in germination and seedling vigour was very slow in KNO_3 invigorated seeds compared to rapid decrease in control and it is due to repairing of damaged membrane system.

Mean seedling length (cm)

The data on mean seedling length (cm) of UARCH-42, PBC-80 and F_1 interspecific hybrid as influenced by seed primed treatments are presented in Table 1, 2 and 3. The mean seedling length differed significantly among different seed priming treatments and it ranged from 10.99 cm to 13.21 cm, 11.32 cm to 9.10 cm and 10.60 cm to 0.00 cm. The highest mean seedling length of 13.21, 11.32 and 10.60 cm was recorded in KNO_3 @ 1per cent, which was on par with 12.95, 11.06 and 0.00 cm in KNO_3 @ 2per cent, followed by 12.63, 10.74 and 0.00 cm in GA_3 @ 50 ppm. Whereas, the control seeds

recorded the lowest mean seedling length of 10.99, 9.10 and 0.00 cm, which was significantly lower than other treatments.

KNO_3 is used as an important seed priming agent in various crop species. Because osmopriming with KNO_3 enhances the seedling performance by increasing its oxidative metabolism by production of super oxidase dismutase (SOD) and peroxidase or by the activation of ATPase and RNAsynthesis this may have led for higher seedling length or shoot length and root length [17]. In this study we have found higher mean seedling length in seeds primed with KNO_3, which was due to production of super oxidase dismutase and peroxidase or by the activation of ATPase and RNA synthesis in the seedlings. Also, the oxidized forms of nitrogen might cause a shift in respiratory metabolism to the pentose phosphate pathway [18]. The results of improvedseedling length by seed priming with KNO_3 were also noticed by [19] in tomato seeds.

Shoot length (cm)

The data on mean shoot length (cm) of UARCH-42, PBC-80 and F_1 interspecific hybrid as influenced by seed primed with dormancy breaking treatments are presented in Table 1, 2 and 3. The mean shoot length differed significantly among different seed priming treatments and it ranged from 5.04 cm to 3.93 cm, 4.49 cm to 3.38cm and4.43 cm and 0.00 cm. The highest mean shoot length of 5.04, 4.49 and 3.38 cm was recorded in KNO_3 @ 1per cent, which was on par with 4.91, 4.36 and 0.00cm in KNO_3 @ 2per cent, followed by 4.75, 4.20 and 0.00 cm in GA_3 @ 50 ppm. Whereas, the control seeds recorded the lowest mean seedling length of 3.93, 3.38 and 0.00cm, which was significantly lower than other treatments and followed by seeds primed with distilled water for 24 hrs (4.10, 3.55 and 0.00 cm), respectively.

Seed invigoration with KNO_3 activated the metabolic activity in the first phase of germination favouring better emergence and more seedling length [20].The beneficial effects of KNO_3@ 3 per cent were attributed to ionic strength and increase in cytochrome oxidase activity. The presence of nitrate in KNO_3 provides additional substrate for accelerated ageing, radicle and plumule emergence and protein synthesis for enhancement of germination during priming and it also helps in membrane repair mechanism [21]. Similar results of higher quality parameters of seeds treated with KNO_3 (3 per cent) were reported by [22] in chilli and [23] in bell pepper.

Root length (cm)

The data on mean root length (cm) of UARCH-42, PBC-80 and F_1interspecific hybrid as influenced by seed primed with dormancy breaking treatments are presented in Table 1, 2 and 3. The mean root length differed significantly among different seed priming treatments and it ranged from 8.17 cm to 7.06 cm, 6.83 cm to 5.72 cm and 6.17 cm to 0.00 cm in case of UARChH-42, PBC-80 and interspecific hybrid respectively. The highest mean root length of 8.17, 6.83 and 6.17 cm was recorded in KNO_3 @ 1per cent, which was on par with 8.04, 6.70 and 0.00 cm in KNO_3 @ 2per cent, followed by 7.88, 6.54 and 0.00 cm in GA_3 @ 50 ppm. Whereas, the control seeds recorded the lowest mean

seedling length of 3.93 cm, which was significantly lower than other treatments and followed by seeds primed with distilled water for 24 hrs (4.10 cm).

The presence of nitrate in KNO_3 provides additional substrate for accelerated ageing, radicle and plumule emergence and protein synthesis for enhancement of germination during priming and it also helps in membrane repair mechanism [21]. Similar results of higher quality parameters of seeds treated with KNO_3 @ 3 per cent were reported by [22] in chilli.

Seedling dry weight (g)

The data on seedlingdry weight (g) of UARCH-42, PBC-80 and interspecific hybrid as influenced by seed treatments are presented in Table 1, 2 and 3. and also represented in Fig. 1, 2 and 3. The seed dry weight differed significantly among the treatments and it ranged from 4.00 to 3.02 g, 3.39 to 2.41 g and 3.22 to 0.00 g for genotypes UARChH-42, PBC-80 and interspecific hybrid respectively.The highest seedling dry weight of 4.00 g, 3.39 g and 3.22 g was recorded when seeds were primed with dormancy breaking treatment @ 1 per cent KNO_3, which was on par with 3.73g, 2.99 g and 0.00 gin KNO_3 @ 2per cent, followed by 3.60 g, 3.12 g and 0.00 g in GA_3 @ 50 ppm. Whereas, the control recorded the lowest seedling dry weight of 3.02 g, 2.41 g and 0.00 g which was significantly lower than other treatments and followed by seeds primed with distilled water for 24 hrs (3.08g, 2.47 g and 0.00 g).

Increased mean seedling dry weight is due to increased seedling length as discussed above. The seeds primed with KNO_3 resulted in rapid germination due to rapid imbibition and revive of seed metabolism with the production of SOD, peroxidase and RNA synthesis and also attributes to the repair mechanism of seeds which result in rapid establishment with higher mean seedling length and thus also leads for higher mean seedling dry weight. The results of improved seedling dry weight by seed priming with KNO_3 was also noticed by [19] in tomato seeds.

Seedling vigour index I and II

The data on seedling vigour index I and II of UARCH- 42, PBC-80 and hybrid as influenced by seed primed with dormancy breaking treatments are presented in Table 1, 2 and 3and also represented in Fig. 1, 2 and 3. The seedling vigour index I and II differed significantly among the seed dormancy breaking treatments and it ranged from 1162.74to 835.47 and 352.00 to 229.52, 894.97 to 628.47 and 267.95 to 166.39, 95.40 to 0.00 and 28.98 to 0.00, in case for genotypes UARChH-42, PBC-80 and interspecific hybrid respectively.

The highest seedling vigour index I and II of 1162.74 and 352.00 of UARCH-42, 894.97 and 267.95 of PBC-80, 95.40 and 28.98 of hybrid was recorded when seeds were primed with dormancy breaking treatment @ 1 per cent KNO_3, which was on par with 1088.05 and 313.32 ofUARCH- 42, 827.64 and 230.35 of PBC-80, 0.00 of interspecific hybrid in KNO_3 @ 2per cent, followed by 1086.44 and 309.60of UARCH- 42, 830.17 and 234.12 of interspecific hybrid in GA_3 @ 50 ppm. Whereas, the control recorded the

lowest seedling vigour index I and II of 835.47 and 229.52of UARCH- 42, 835.47 and 229.52 of PBC-80, 0.00 of interspecific hybrid which was significantly lower than other treatments and followed by seeds primed with distilled water for 24 hrs (883.97 and 240.24of UARCH- 42, 661.01 and 172.90 of PBC-80, 0.00 of interspecific hybrid), respectively.

Seedling vigour index I and II is a resultant of germination and seedling length. Increased seedling length and seed germination was observed in seeds primed with KNO_3 in turn resulted in higher vigour index. Such increased vigour index due to osmopriming with KNO_3 was also noticed by [24] in brinjal seeds.Increase in seed quality parameters may be due to enlarged embryos, higher rate of metabolic activity and respiration, better utilization and mobilization of metabolites to growing points and higher activity of enzymes. The superiority of KNO_3 @ 3 per cent ascribed to its role in making less oxygen available for the citric acid cycle and thereby enhancing the ambient oxygen level. [25]. The increase in seedling vigour index and seedling dry weight was due to increased germination percentage, root length and shoot length of seedlings.

Conclusion

Due to poor germination in interspecific F_1 hybrid seeds of chilli. Some strategies have been used in order to improve seed germination. Among the strategies to improve the germination in interspecific F_1 hybrid seeds chemicals like KNO_3 and GA_3 at different concentrationswhich were used extensively in solanaceous crops. Among them, KNO_3 at 1% which was on par with KNO_3 @ 2 % and followed by GA_3 at 50 ppm against UARChH-42, PBC-80 and Interspecific F_1 hybrid recorded statistically significant in parameters such as germination per cent, radical length, plumule length, seedling length, dry weight and seed vigour index I and II.

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Table 1. Effect of seed treatment on germination and growth parameters in chilli (UARCH-42)

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seedling dry weight (g)	SVI – I	SVI - II
Control	76.00	7.06	3.93	10.99	3.02	835.47	229.52
Water	78.00	7.23	4.10	11.33	3.08	883.97	240.24
1% KNO_3	88.00	8.17	5.04	13.21	4.00	1162.74	352.00
2% KNO_3	86.00	8.04	4.91	12.95	3.73	1088.05	313.32
3% KNO_3	80.00	7.42	4.29	11.71	3.41	937.04	272.80
50 PPM GA_3	84.00	7.88	4.75	12.63	3.60	1086.44	309.60
100 PPM GA_3	83.00	7.39	4.26	11.65	3.26	967.20	270.58
150 PPM GA_3	81.00	7.23	4.10	11.33	3.19	917.97	258.39
S.Em	2.23	0.17	0.09	0.28	0.08	13.46	7.00
CD 1 %	9.20	0.69	0.36	1.16	0.35	55.58	28.89

SVI – I = Seed vigour index I

SVI – II = Seed vigour index II

Table 2. Effect of seed treatment on germination and growth parameters in chilli (PBC-80)

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seedling dry weight	SVI I	SVI II

					(g)		
Control	69.04	5.72	3.38	9.10	2.41	628.47	166.39
Water	70.00	5.89	3.55	9.44	2.47	661.01	172.90
1% KNO_3	79.04	6.83	4.49	11.32	3.39	894.97	267.95
2% KNO_3	77.04	6.70	4.36	11.06	2.99	827.64	230.35
3% KNO_3	71.04	6.08	3.74	9.82	2.80	697.83	198.91
50 PPM GA_3	75.04	6.54	4.20	10.74	3.12	830.17	234.12
100 PPM GA_3	74.04	6.05	3.71	9.76	2.65	722.85	196.21
150 PPM GA_3	72.04	5.89	3.55	9.44	2.58	680.27	185.86
S.Em	1.60	0.12	0.10	0.25	0.06	16.86	4.76
CD 1 %	6.59	0.50	0.42	1.04	0.26	69.65	19.67

SVI – I = Seed vigour index I

SVI – II = Seed vigour index II

Table 3. Effect of seed treatment on germination and growth parameters in chilli F_1 interspecific hybrid (UARCH42 × PBC80)

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seedling dry weight (g)	SVI I	SVI II
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Water	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1% KNO_3	9.00	6.17	4.43	10.60	3.22	95.40	28.98
2% KNO_3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3% KNO_3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
50 PPM GA_3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
100 PPM GA_3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
150 PPM GA_3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S.Em	0.02	0.01	0.02	0.03	0.01	0.28	0.06
CD 5%	0.06	0.06	0.06	0.14	0.04	1.14	0.24

SVI – I = Seed vigour index I

SVI – II = Seed vigour index II

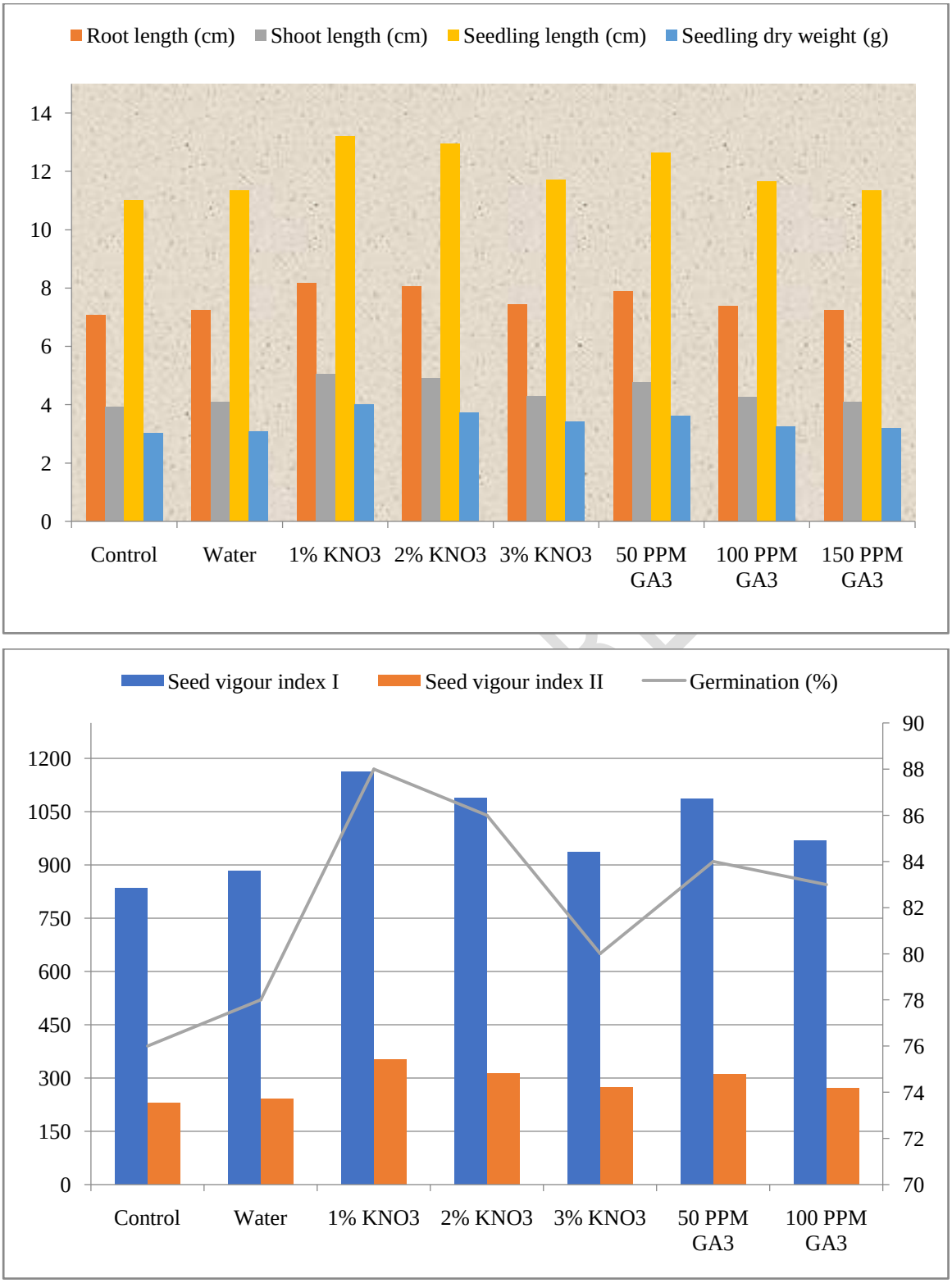
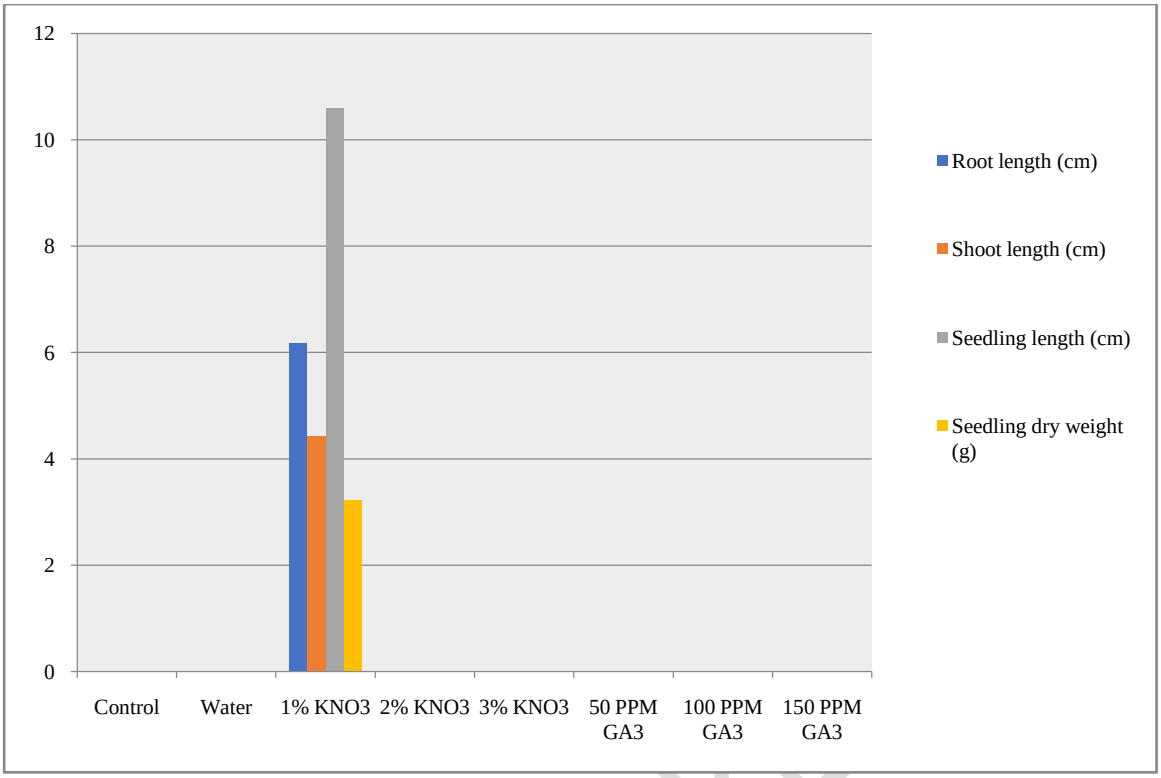


Fig. 1. Effect of seed treatment on germination and growth parameters in chilli UARCH-42

Fig. 2. Effect of seed treatment on germination and growth parameters in chilli PBC-80



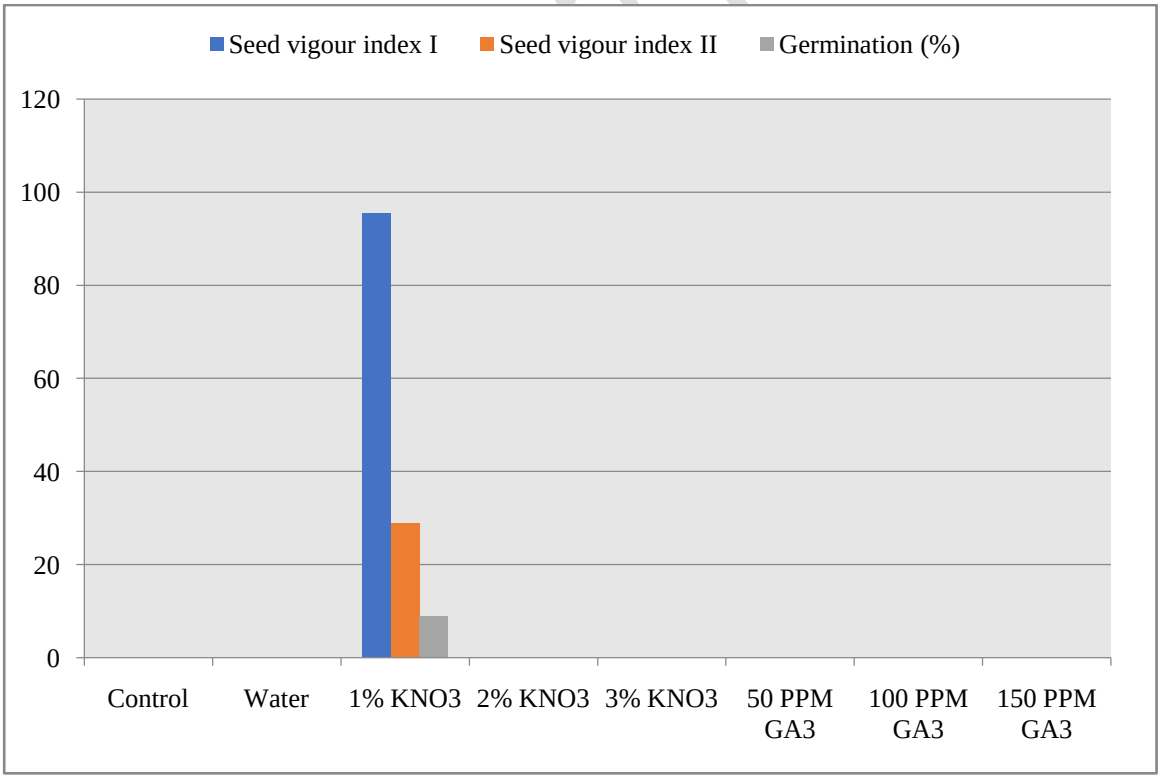


Fig. 3. Effect of seed treatment on germination and growth parameters in chilli F_1 interspecific hybrid