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Growth and Productivity of Cowpea (*Vigna unguiculata* L.) in Response to Seed Priming and Different Levels of Phosphorus

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ABSTRACT

Acidic soil generally has lower availability of phosphorus. Grain legumes respond positively to higher levels of phosphorus in acidic soil. Seed priming also enhances growth of the crops by activating various metabolic processes in plants. A field experiment was carried out to determine the influence of seed priming and phosphorus levels on growth and productivity of cowpea at Bhojad, Chitwan, Nepal. The experiment plot was laid out in two factorial randomized complete block design with three replications and the planting was done in spring season of 2022. The treatment consisted of two levels of seed priming (no priming and priming with 0.2 % $Ca(NO_3)_2$ for 24 hours) and four levels of P_2O_5 (20, 30, 40 and 60 kg ha^{-1}). The variations in different parameters of cowpea due to seed priming were significant ($P < 0.05$). Primed seed produced the highest grain yield (1.25 t ha^{-1}), which was followed by non-primed seeds (1.16 t ha^{-1}). Similar significant ($p<0.001$) differences in yield and yield-attributing (no. of grains/pods, test weight, biological yield, grain yield and harvest index) traits were found at different levels of P_2O_5, with the highest grain yield (1.44 t ha^{-1}) was obtained when applied with 40 kg P_2O_5 ha^{-1}. Significant ($p<0.001$) variations in grain yield were found as a result of the interaction between priming and P_2O_5 levels. The combination of priming and 40 kg P_2O_5 ha^{-1} produced the highest grain yield (1.51 t ha^{-1}). The results showed that the use of primed seed with 40 kg P_2O_5 ha^{-1} would be beneficial for the farmers in the Terai region of Nepal.

Keywords: Calcium nitrate, cowpea, biological yield, harvest index, growth

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INTRODUCTION

Cowpea (*Vigna unguiculata* L.), an annual herbaceous plant of the Fabaceae family, is cultivated as an edible legume in tropical and subtropical locations all over the world. They are also frequently grown as a hay crop, green manure, or cover crop and are recognized as a food crop high in protein (Britannica 2022). Cowpea is an excellent source of fiber, protein, carbs, carotene, and several different minerals (Arun 2017). Due to the high protein content of cowpea seed and leaves, it is often referred to as "poor man's meat." Vegetables for human consumption can be obtained from fresh and immature pods, leaves, and green cowpea seeds (Gerrano 2017). Although gross area of cowpea cultivation is estimated to be 12.5 million hectares, over the world more than 3 million tons of cowpea is produced (Kaur 2020). According to FAOSTAT (2019) and Samireddypalle (2017), Nigeria is emerged as the leading producer and consumer of cowpea with 3.4 million of production and also more than 95% of world's production is covered by West Africa. In Nepal, pulse crops cover an area 335143 ha and production and productivity of 394355 metric (Krishi Diary 2079). Cowpea is beneficial as a cover crop that prevents erosion, eliminates weeds, and also helps retain moisture (Das 2018). When grown in a rotation with cereal crops, cowpea is recognized as a crucial crop that supplies nitrogen to those crops (such as maize, millet, and sorghum).

Legumes have an ability to fix atmospheric nitrogen, an essential element for the production of cereals can be offered to cereal crops when they are grown in rotation is (Shen 2004). It also has been noticed that among leguminous crops, cowpea is the topmost crop to enhance the soil fertility (IITA 1990). Being a leguminous crop, cowpea forms nodules in its roots which help to fix nitrogen and enhance the soil fertility. As legumes are phosphorus loving plants; they require phosphorus mostly in nitrogen fixation which is an energy-driving process and also required for seed development (Nkaa 2014). The main reasons for the low productivity of cowpea that is observed in tropical soils are deficiencies in phosphate and nitrogen (Haruna 2011). Soil fertility and soil quality is declining due to the recent changes in agricultural practices (Bista 2010). The soil fertility has also been diminished by farmers' inappropriate application of the recommended fertilizer amounts. Phosphorus is regarded as the second-most important nutrient in soil because it helps with the uptake of nitrogen fertilizer and promotes nodule growth, which in turn enhances N_2 fixation and cowpea yield (Singh et al 2011). It is suggested that cowpea cultivation employ roughly 30 kg of P_2O_5 ha^{-1} in the form of super phosphate in order for the crop to nodulate well and fix its own nitrogen from the atmosphere (FAO 2005). According to (Singh, ., 2011), cowpea requires phosphorus, although not in large quantities for the better yield. Seed priming can be described as a process of controlled hydration and drying which results in more germination when the seed is reimbibed (Gurusinghe 1999). According to Marcos Filho (2015), osmo-priming using chemicals like polyethylene glycol (PEG), calcium nitrate, and potassium nitrate activates seed germination by creating a water potential balance between the seeds and the solution. These substances, which are also less expensive and well-known chemicals, have a strong capacity for dissolving. Water stress is considered as one of the factors which limit the cowpea production, due to its effect on whole process of growth of plant organs and its metabolism (Zimmermarin 1988). Thus, to stimulate such metabolic processes involved in the early phases of germination, priming can be one of the appropriate solutions (Arun 2020).

Therefore, the research prioritized to evaluate the impact of seed priming and phosphorus doses on yield and yield-attributing characteristics of cowpea.

MATERIALS AND METHODS

Experimental location, soil properties and climatic status

The current investigation was conducted in the Nepal Polytechnic Institute's (NPI) agronomy farm in Bharatpur 11, Bhojad, and Chitwan during the months of Falgun 2078 and Jestha 2079. The precise coordinates of location were 27° 70' N latitude, 84° 45' E longitude, and its elevation was 250 m above mean sea level. To determine the soil pH, available plant nutrients, and organic matter percentage, a soil sample was taken from the experimental field at a depth of 15-20 cm prior to the experiment.

Table 1: Initial physio-chemical properties of the soil in the experimental field

Details	Mean	Rating
pH	5.65	Acidic
Organic Matter (%)	4.72	Medium
Total Nitrogen (%)	0.61	Very high
Available P_2O_5 (kg ha^{-1})	17.41	Medium
Available K_2O (kg ha^{-1})	70.08	Low

Soil Organic matter, total nitrogen, available P_2O_5 and available K_2O were rated according to the rating chart provided by Nepal Agricultural Research Council (NARC 2013) and soil pH was rated by rating chart given by (Khatri Chhetri 1991) and (Jaishy et al 2000).

Table 2: Meteorological data of the experimental location in 2022

Months	Temperature (ºC)		Total rainfall (mm	Relative humidity (%)
	Minimum	Maximum		
February	7	24	38.66	46
March	11	29	49.9	35
April	14	32	165.24	37
May	16	33	344.19	54

Description of experimental materials, design, treatment details and cultural practices

For the experiment, the cowpea variety "Prakash" was used. The seeds were purchased from National Grain Legumes Research Program, Khajura, Nepalgunj. For seed priming, calcium nitrate was employed, and it was purchased from CMI Scientific Traders in Narayangarh, Chitwan, Nepal.

The design of experiment consisted of two-factorial randomized complete block design with three replications. The treatments were factor A: priming (a) non-priming and (b) priming (with 0.2% Ca $(NO_3)_2$ for 24 hours) and factor B: phosphorus levels (20, 30, 40, and 60 kgha^{-1} P_2O_5). Seed rate was 20 kg ha^{-1} in which half of the seeds (150g) were primed with 0.2% Ca $(NO_3)_2$ for 24 hours and half were not primed. Meanwhile, 24 plots were prepared with each plot size of 3m by 2m. Just prior to sowing, seeds were treated with Bavistin at the rate of 2.5 g kg^{-1} of seed, and they were then planted using the dibbling method with two seeds per spot at a depth of 4-5 cm in 60 cm (RR) × 20 cm (PP) spacing. The recommended doses of phosphorus (20 kg ha^{-1}, 30 kg ha^{-1}, 40 kg ha^{-1}, and 60 kg ha^{-1}) as well as nitrogen and potassium (20:20 kg ha^{-1}) was applied as basal dose. After 30 days after sowing (DAS) half of the N was applied as the first top dressing. Single superphosphate (16% P_2O_5) was and muriate of potash (60% K_2O) was used to supply phosphorus and potassium respectively. After 10 DAS, gap filling was carried out in order to maintain the plant population in the plot. Two manual weeding were done, the first at 30 DAS and the second at a 20-day interval. Pods produced from each net plot were then harvested and cleaned through winnowing. Crop plants were harvested and allowed to dry in the field for three to four days. Then, the weighted biological yield included the entire plant, including the pods.

Table 3: Details of factors used in treatments in the experiment

Seed priming (P)	Fertilizer P_2O_5 levels (F)
Non primed seed (P_1)	20: 20: 20 kg N: P_2O_5: K_2O ha^{-1} (F_1)
Primed seed with 0.2% Ca $(NO_3)_2$ (P_2)	20: 30: 20 kg N: P_2O_5: K_2O ha^{-1} (F_2)
	20: 40: 20 kg N: P_2O_5: K_2O ha^{-1} (F_3)
	20: 60: 20 kg N: P_2O_5: K_2O ha^{-1} (F_4)

Observations and data collection

Ten plants per plot were randomly selected to collect data for all the parameters. Plant height (cm), number of green leaves per plant, pod length (cm), number of grains per pod, test weight (grams), grain yield and biomass (t ha^{-1}), and the harvest index (%) were measured.
Following formula were used to calculate different parameters:
Grain yield: -
Grain yield at 10% moisture was calculated using following formula,

$$\text{Grain yield (kg ha}^{-1}\text{) at 10\% moisture} = \frac{(100 - \text{MC}) \times \text{Plot yield (kg)}}{(100 - 10) \times \text{Plot area (m}^2\text{)}} \times 10000\text{m}^2 \qquad \text{........Eq.(1)}$$

Where, MC= moisture content (measured through moisture meter)

Harvest Index: -
Harvest index was calculated as:

$$\text{Harvest Index} = \frac{\text{Grain yield (kg ha}^{-1}\text{)}}{\text{Biological yield (kg ha}^{-1}\text{)}} \times 100 \qquad \text{..............................Eq.(2)}$$

Statistical analysis

Data recorded was systematically arranged in MS-Excel (16.0), which was used for simple statistical analysis, constructing graphs and tables. Then, the compiled data were subjected to analysis of variance (ANOVA) using R-studio statistical packages (1.0.153). ANOVA was constructed and significant were subjected to DMRT (Duncan's Multiple Range Test) at 5% level of significance for mean separation.

RESULTS

Growth traits

The effect of seed priming on cowpea growth traits including plant height (cm), the number of green leaves per plant, and pod length (cm), as influenced by different phosphorus doses, are shown in Table 4. Plant height and the number of green leaves per plant, were significantly influenced but pod length exhibited non-significant changes. Higher plant height (47.21 cm), number of green leaves plant^{-1} (25), and pod length (17.96 cm) were found to be superior when Ca $(NO_3)_2$ priming was compared to non-priming.

Table 4: Growth characters of cowpea as influenced by seed priming and different phosphorus levels at Bharatpur, Chitwan, Nepal in 2022

Treatments	Plant height (cm)	No. of green leaves plant^{-1}	Pod length (cm)
Priming level			
P_1: Non- Priming	44.86^{b}	22^{b}	16.99
P_2: Priming	47.21^{a}	25^{a}	17.96
Grand mean	46.03	25	17.48
SEm (±)	0.09	0.06	0.07
LSD (0.05)	1.97	1.29	1.51
F test	*	*	NS
Phosphorus level			
F_1:20:20:20 kg N: P_2O_5: K_2O ha^{-1}	44.01^{c}	23^{b}	16.42^{c}
F_2:20:30:20 kg N: P_2O_5: K_2O ha^{-1}	45.37^{bc}	23^{b}	17.18^{b}
F_3:20:40:20 kg N: P_2O_5: K_2O ha^{-1}	48.28^{a}	26^{a}	18.38^{a}
F_4:20:60:20 kg N: P_2O_5: K_2O ha^{-1}	46.47^{b}	23^{b}	17.93^{a}
Grand mean	46.03	24	17.48
SEm (±)	0.20	0.13	0.07
LSD (0.05)	1.51	1.01	0.56
F test	***	***	***

Mean followed by the same letter(s) in a column do not differ at 5% level of significance by DMRT. LSD: Least significant difference, SEm (±): Standard error of mean, *P<0.05, ***P<0.001, NS: Non-significant.

Cowpea growth characteristics were also significantly affected by phosphorus levels, with superior plant height (48.28 cm), number of green leaves per plant (26), and pod length (18.38 cm) being reported in the 40 kg P_2O_5 ha^{-1} treated plot. Similar results were seen in 20 kg P_2O_5 ha^{-1} for lower plant height (44.01 cm), number of green leaves plant^{-1} (23), and pod length (16.42 cm).

Yield and yield attributing characters

The relationship between yield-attributing characteristics, such as the number of grains pod^{-1} and test weight (g) of cowpea due to seed priming and various phosphorus levels are given in Table 5. Data analysis showed that test weight was considerably influenced by the effects of priming and phosphorus doses, but the number of grains pod^{-1} exhibited non-significant changes due to the effect of priming but was strongly influenced by the effect of phosphorus levels. The Ca $(NO_3)_2$ priming process had the highest number of grains pod^{-1} (15), which was statistically equivalent to non-priming. Ca $(NO_3)_2$ priming also had the highest test weight (108.94 g). In terms of factor B phosphorus doses, the highest number of grains per pod (15) and highest test weight (113.39 g) were found in the application of 40 kg P_2O_5 ha^{-1}, while the lowest number of grains per pod (14) and lowest test weight (101.55 g) were found in the application of 20 kg P_2O_5 ha^{-1}.

Table 5: Yield and yield attributing characters of cowpea as influenced by seed priming and different phosphorus levels at Bharatpur, Chitwan, Nepal in 2022

Treatments	No. of grains pod^{-1}	Test weight (g)	Biological yield ($t\ ha^{-1}$)	Grain yield ($t\ ha^{-1}$)	Harvest Index (%)
Priming level					
P_1: Non- Priming	15	105.50^{b}	4.98	1.16	23.11
P_2: Priming	15	108.94^{a}	5.14	1.25	24.22
Grand mean	15	107.28	5.06	1.20	23.67
SEm (±)	0.01	0.01	0.01	0.01	0.31
LSD (0.05)	0.15	0.21	0.26	0.27	6.58
F test	NS	***	NS	NS	NS
Phosphorus level					
F_1:20:20:20 kg N: P_2O_5: K_2O ha^{-1}	14^{c}	101.55^{d}	4.48^{c}	0.93^{b}	20.70^{b}
F_2:20:30:20 kg N: P_2O_5: K_2O ha^{-1}	15^{b}	104.01^{c}	5.19^{b}	1.09^{b}	21.02^{b}
F_3:20:40:20 kg N: P_2O_5: K_2O ha^{-1}	15^{a}	113.39^{a}	5.39^{a}	1.44^{a}	26.84^{a}
F_4:20:60:20 kg N: P_2O_5: K_2O ha^{-1}	15^{a}	109.94^{b}	5.19^{b}	1.35^{a}	26.11^{a}
Grand mean	15	107.22	5.06	1.20	23.67
SEm (±)	0.02	0.31	0.02	0.03	0.57
LSD (0.05)	0.18	2.37	0.15	0.24	4.34
F test	***	***	***	**	*

Mean followed by the same letter(s) in a column do not differ at 5% level of significance by DMRT. LSD: Least significant difference, SEm (±): Standard error of mean, *P<0.05, **P<0.01, ***P<0.001, NS: Non-significant

The yield of cowpea, including its biological yield, grain yield, and harvest index, as affected by seed priming and various phosphorus levels, is also presented in Table 5. Grain yield, biological yield and the harvest index were not considerably impacted by the priming effect. Ca $(NO_3)_2$ priming increased biological yield (5.14 t ha^{-1}), grain yield (1.25 t ha^{-1}) and harvest index (24.22%) compared to non-priming.

The effect of phosphorus levels on yield revealed significant differences, with the 40 kg P_2O_5 ha^{-1} applied plot recording the highest biological yield (5.39 t ha^{-1}), grain yield (1.44 t ha^{-1}), and harvest index (26.84%), whereas the 20 kg P_2O_5 ha^{-1} application recorded the lowest biological yield (4.48 t ha^{-1}), grain yield (0.93 t ha^{-1}), and harvest index (20.70%).

Interaction effects of seed priming and phosphorus levels on yield and yield attributing characters of cowpea

The effects of seed priming and various phosphorus levels on the production and yield characteristics of cowpea in Bharatpur, Chitwan, in 2022 are discussed in Table 6.

Table 6: Interaction effects of seed priming and different phosphorus levels on yield and yield attributing characters of cowpea at Bharatpur, Chitwan, Nepal in 2022

Interaction effects	No. of grains pod^{-1}	Test weight (g)	Biological yield (tha^{-1})	Grain yield ($t\ ha^{-1}$)	Harvest Index (%)
$P_1 \times F_1$	14	100.88	4.49	0.84	18.83
$P_1 \times F_2$	15	103.07	5.22	1.03	19.83
$P_1 \times F_3$	15	109.95	5.30	1.20	22.69
$P_1 \times F_4$	15	108.12	5.16	1.17	22.74
$P_2 \times F_1$	15	102.23	4.71	1.01	21.44
$P_2 \times F_2$	15	104.61	5.16	1.08	20.93
$P_2 \times F_3$	15	116.83	5.49	1.51	27.62
$P_2 \times F_4$	15	112.10	5.22	1.40	26.91
Grand mean	15	107.22	5.09	1.15	22.62
SEm (±)	0.05	0.62	0.04	0.064	1,15
LSD (0.05)	0.26	3.36	0.21	0.34	6.13

LSD: Least significant difference, SEm (±): Standard error of mean, *P<0.05, NS: Non-significant.

The interaction effect of seed priming and various phosphorus doses revealed that treatments had no statistically significant effect on the number of grains in each pod^{-1}, test weight, grain yield and harvest index but did have an effect on biological yield. Comparatively, primed seeds with 40 kg of P_2O_5 ha^{-1} produced the highest number of grains pod^{-1} (15), test weight (116.83 g), biological yield (5.49 t ha^{-1}), grain yield (1.52 t ha^{-1}), and harvest index (27.62%), while non-primed seeds with 20 kg of P_2O_5 ha^{-1} produced the lowest number of grains pod^{-1} (14), test weight (100.88 g), biological yield (4.49 t ha^{-1}), grain yield (0.84 t ha^{-1}) and harvest index (18.83%).

DISCUSSION

Effect of seed priming on growth characters, yield and yield attributing characters of cowpea

In addition to promoting cell growth and elongation, calcium nitrate priming also affects the stability and permeability of cell membranes (Marschner 1995, Ashraf 2004). This conclusion was supported by Arun (2017), who discovered that cowpea pod length increased with GA_3 priming (100 ppm). This result closely matched with findings of Singh (2014) which showed an increase in cowpea plant height after Osmopriming for 6, 8, and 10 hours. In contrast to control and hydropriming, Arun (2020) noticed an increase in plant height, the number of trifoliate leaves, LAI, RGR, CGR, NAR, grain yield, and biological yield in cowpea seeds that had undergone chemical priming. According to Maroufi et al (2011), cowpea seedling vigor and dry weight were at their highest levels after 6 hours of hydroponic priming. The findings of Ali and Kamel (2009), who discovered a larger plant height and the highest number of trifoliate leaf plants (1 in chickpea) by different priming strategies, are consistent with the finding that seed priming improved plant height when compared to un-primed plants.

Effect of phosphorus levels on growth characters, yield and yield attributing characters of cowpea

The results presented in Table 4 and 5 indicate that the application of phosphorus has a significant influence on the growth characteristics, yield and yield attributing characters of cowpea. Highest values for these parameters were observed when 40 kg P_2O_5 was applied. As cowpea is known to be phosphorus-loving plant, it requires an adequate supply of phosphorus for optimal growth and development. In the experimental field where the study was conducted, availability of phosphorus was determined to be medium to low. Also, soil was acidic in the research field which makes lower availability of phosphorus. This indicates that the natural levels of phosphorus in the soil were not sufficient to meet the crop's demand which is why additional phosphorus had to be applied to the cowpea crop. Nkaa et al (2014) observed that plant height of cowpea and number of leaves per plant was significantly enhanced by phosphorus application. This result was closely in line with Ndor's (2012) conclusion that the application of 40 kg ha^{-1} of phosphorus fertilizer results in the highest number of leaves (20.81 and 24.82), branches (7.85 and 7.95), vine length (90.02 and 78.00 cm), root nodules (34.95 and 32.24), pods (20.64 and 20.24), seed weight plant^{-1} (39.56 and 37.64g), pod weight plant^{-1} (51.45 and 45.31g), and Jat (2013) confirmed this finding by observing greater plant height of cowpea on 40 kg P_2O_5 ha^{-1} application over 20 kg P_2O_5 ha^{-1} and control. This result is quite similar to that of Namakka (2017) and Nkaa et al (2014), who found that 39 kg of P_2O_5 ha^{-1} generated considerably ($P<0.05$) higher plants and more leaves than the control. Subbarao (2014) also came to the same conclusion, noting that phosphorus application considerably raises the quantity of leaves and plants as well as the leaf area index. The obtained results are more in line with Okeleye and Okelana's (1997) observations, which showed that adding P_2O_5 to cowpea cultivars greatly boosted their grain yield and total dry matter. Furthermore, Choudhary (2011) confirms his finding that foliar spraying with 2% DAP, urea, and KCl resulted in significantly higher straw and biological yields, as well as harvest index, when compared to water spraying. Magani (2009) also gives close conformity to this result by concluding that an application of 37.5 kg ha^{-1} was the most economical level for maximum grain yield and crude protein content. The results obtained are inconsistent with the result obtained by Namakka (2017), who concluded that an application of 39 kg ha^{-1} under rain-fed conditions in well-drained sandy loam soil resulted in the highest growth and yield of the SAMPEA 17 cowpea variety. These findings suggest that 40 kg P_2O_5 application in cowpea were able to achieve superior growth parameters, yield and yield attributing characters.

Interaction effects of seed priming and phosphorus levels on yield and yield attributing characters of cowpea

Calcium is crucial in reducing the negative effects of salinity on plants (Ehret et al 1990). According to Cramer et al (1986), calcium is a metabolic regulator. Osmopriming with calcium nitrate hydrates seeds while waiting for the solution to achieve equilibrium and the biochemical germination process to start (Marcos Filho 2015). Additionally, it can be used to break dormancy in some seeds and to pre-treat seeds to speed up germination (Silva et al 2017). According to Rahman et al (2008) and Niu et al (2012), phosphorus is an element that contributes to the development of lateral, fibrous, and adventitious roots as well as nodule formation, N_2 fixation, nutrition, and water uptake. Furthermore, Ousman (2011) found that the interaction between seed priming and 0.3 g of fertilizer enhanced cowpea yield by 54.5% when compared to the control.

CONCLUSION

The priming cowpea seeds with 0.2% calcium nitrate solution for 24 hours improved several growth parameters, yield-attributing traits, and yield compared to unprimed seeds. Similarly, phosphorus level @40 kg P_2O_5 ha^{-1} was found suitable dose to improve several growth, yield-attributing traits, and yield of cowpea. Among the combinations of seed priming and different phosphorus levels, the use of 0.2% $Ca(NO_3)_2$ solution along with 40 kg P_2O_5 ha^{-1} showed the highest vegetative growth parameters. The study found that cowpea performed better in 0.2% $Ca(NO_3)_2$ solution with 40 kg P_2O_5 ha^{-1} to increase the yield of cowpea in spring season at Bharatpur, Chitwan in comparison to other treatments.

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AUTHORS' CONTRIBUTION

Asmita Amgai: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper

Bishnu Bilas Adhikari: Regular constructive comments and support during this research work; guidance during preparation of manuscript.

Jiban Shrestha: Revised the initial draft of the paper; wrote the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interests or personal relationships that could have appeared to influence the work reported in this paper.

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