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Effect of seed priming on different physiological parameters of Cowpea (*Vigna unguiculata* L. Walp) seeds collected from Western Odisha

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Abstract
A study was conducted with ten cowpea genotypes which were collected from farmer's of different districts of Western Odisha. Those seeds which didn't meet the minimum seed germination percentage as prescribed by the IMSCS were further taken for seed priming treatments to improve the quality of the cowpea seeds. The experiment was conducted in completely randomized design using 14 treatments with four replications. The treatment consists of; Control (no priming), Deionised water, KNO_3@ 0.5%, KNO_3 @ 1%, KCl @ 1%, KCl @ 2%, *Trichoderma viride* @10gram/ kg seed, *Pseudomonas fluorescence* @10gram/ kg seed, GA_3 @ 50ppm, GA_3 @ 100ppm, Ascorbic acid @ 100ppm, Ascorbic acid @ 150ppm, 10^{-2}M Ammonium Molybdate, 10^{-3}M Ammonium Molybdate. The cowpea seeds were soaked for 6 hours and then dried back to original moisture content. Kantamal, Boudh collected genotype performed best in terms of germination and seedling development, followed by Rupra road, Kalahandi genotype and Rairakhol, sambalpur genotype of farmer saved seed. Seed priming treatments with KCl @ 1%, KNO_3 @ 1% and *Trichoderma viridae* @ 10g/kg seeds were found to be superior to seed germination percentages that met the minimum seed certification standards for the cowpea seed germination as well as treatments with GA_3 @ 50ppm (hormonal priming) and ammonium molybdate @ 10-^{3}M (nutri-priming) that also improved seed germination and seedling growth in farmer-saved cowpea seeds.

Keywords: Seed priming, hydropriming, osmopriming, hormonal priming, biopriming, nutripriming

Introduction
Cowpea is an annual herbaceous leguminous crop belonging to family Fabaceae. Its origin is West Africa whereas it came to India around 200BC. It is a versatile crop because of which is regarded as multifunctional crop. Cowpea can be grown for its grain, pod as vegetable, leaves as fodder, hay, silage, mulching material, intercrop with many cereal crops, fixes atmospheric nitrogen and many more. The protein content is around 22.4%, carbohydrate 55-66%, iron levels varied from 2.0 to 2.4 mg/1kg seeds, whereas calcium levels ranged from 9 to 36 mg/100g (Gondwe *et al.*, 2019) [9]. It also contains vitamins such as thiamine (vitamin B1), riboflavin (vitamin B2), and niacin (vitamin B3) (vitamin B3). It also has lysine, leucine, and phenylalanine, among other vital amino acids.
Despite of all these in many developing countries like India the production is very low. The reason may be the lack of knowledge among farmers about the nutritional qualities, production practices, lack of handling the seeds after harvesting etc. For agricultural purposes, farmers in these different sampling sites are using their own stored seeds as well as locally obtained seed. Many researchers indicated that the seed quality of farmer-saved seed does not reach the seed standard for the crops in their evaluations (Eskandari and Kazemi 2011; Kamara *et al.*, 2019 and Njonjo *et al.*, 2019) [7, 11, 15]. Seed priming treatments may be recommended in this situation to promote germination, speed of germination, seedling vigour and reduce emergence time. One of the strategies for improving the quality of low vigour seed before planting is seed priming. It is controlled by hydrating seeds to a point where pre-germinative metabolic activity may continue but radicle emergence is prevented. Application of Gibberellic Acid (GA_3) has been reported to increase germination percentage and seedling growth of crop plants under salt stress (Biswas *et al*., 2020) [4]. The influence of GA_3 has been found to enhance seedling growth of crop plants (Ray and Bordolui, 2020, Biswas *et al*., 2021) [4, 3]. Seed priming with different priming methods like hydropriming, osmopriming, hormonal priming, nutripriming, nano priming etc. can be done to improve the germination, uniform seedling establishment as these are essential stage of a plant life.

Therefore, the study was thus undertaken to evaluate the effect of different seed priming treatments on farmer's saved cowpea seed towards its germination and seedling growth.

Materials and Methods

This study was carried out in the Department of Seed Science and Technology College of Agriculture, Bhawanipatna, OUAT during 2020-21. The materials of this study comprised of ten cowpea genotypes which were collected from farmers of different districts of Western Odisha The experiment was conducted in completely randomised design with four replications. A total of fourteen treatments were taken for priming in which seeds are primed for 6 hours and then dried back to original moisture content. These priming treatments were- T_0- Control (unprimed), T_1- Hydropriming (Soaking of seeds in de-ionised water for 6 hours), T_2- Osmopriming (KNO_3@ 0.5%, T_3- KNO_3 @ 1%, T_4- KCl @ 1%, T_5- KCl @ 2%), T_6- Biopriming (*Trichoderma viride* @10gram/ kg seed, T_7- *Pseudomonas fluorescence* @10gram/ kg seed), T_8- Hormonal priming (GA_3 @ 50ppm, T_9- GA_3 @ 100ppm, T_{10}- Ascorbic acid @ 100ppm, T_{11}- Ascorbic acid @ 150ppm), T_{12}- Nutripriming (10^{-2}M Ammonium Molybdate and T_{13}- 10^{-3}M Ammonium Molybdate). Observations were taken regularly until final germination count was recorded. First and final count was taken in 5th and 8th day respectively. Out of ten only four genotypes were taken those didn't meet the IMSCS standard for germination percentage. The genotypes taken for priming were- Rairakhol, Sambalpur genotype (G_1), Kantamal, Boudh genotype (G_2), Rupra road, Kalahandi genotype (G_3) and Khariar, Nuapada genotype (G_4). All the recorded data are studied in two factorial analysis with factorial design having 14 treatments, 4 replications and 4 genotypes. The different seed quality parameters such as root length, shoot length, dry weight, germination percentage and vigor index were recorded. Germination test was carried out using glassplate and petri-plate method (ISTA, 1985) and calculated as Germination (%) = No. of normal seedlings germinated × 100/ Total no. of seeds placed for germination.
Speed of germination was calculated according to the equation of Ellis and Roberts (1981) [6]:

$$MGT = \frac{\sum Dn}{\sum n}$$

Where, n indicates the number of seeds, which were germinated on day D, and D is the number of days counted from the beginning of germination. Root length and shoot length test was carried out by glassplate method. Vigor Index was also calculated by Abdul-Baki and Anderson (1973) [1] as
Vigor index-I = Germination (%) × Seedling length (cm).
Vigor index -II= Germination (%) × Seedling dry weight (g).

Results and Discussion

Germination percentage

Seed priming treatments demonstrated a substantial variation in germination percentage in this study (Table-1). Seed germination percentage and speed increased after osmopriming with KCl and KNO_3, hormonal priming with GA_3 and ascorbic acid and nutripriming with ammonium molybdate @10^{-3}M for all genotypes collected from various locations in Western Odisha, whereas non-primed seeds had the lowest germination percentage. For germination, the interaction impact between the genotypes and treatments was shown to be significant. Maximum germination was obtained in combinations of Rupra road, Kalahandi genotype with KCl @1% (85%), followed by Rairakhol, Sambalpur genotype with KCl @1% (82%) for this parameter which is statistically significant.

Speed of germination

In this study, GA_3 @50ppm hormonal priming increased the rate of germination and the proportion of seeds that germinated (Arun *et al.*, 2017, Das *et al.*, 2014 and Faruk, 2015) [2, 5, 8] (Table-2). Gibberellins promote germination by inducing dormancy and germination by activating enzymes that weaken tissue barriers such as endosperm or integuments, mobilise seed storage and increase embryo growth. The present findings are in accordance with the findings of Arun *et al.*, (2017) [2] in cowpea. In the instance of biopriming, the effect of *Trichoderma viridae* and *Pseudomonas florescence* on various farmer's seed showed a boost in germination percentage and speed of germination, both of which had a statistically equivalent response to germination percentage. Mohamedy *et al.*, (2006) [14] found similar type findings in cowpea while Sharma *et al.*, (2018) [17] reported similar in soybean seeds.

Root and shoot length (cm)

Regardless of cowpea variety, seed priming treatments had a substantial impact on root and shoot length. KNO_3 @1%, KCl @1%, GA_3@50ppm, and ammonium molybdate @ 10^{-3}M were shown to have the maximum mean root and shoot lengths among the treatments (Table 3). The seed priming treatments resulted in longer seedlings than the control. In comparison to control, Faruk (2015) [8] observed in lentil seeds treated with KNO_3 enhanced germination rate, germination percentage, root and shoot dry weight, number of nodules, yield component and grain yield. The GA_3 treatment promotes the hydrolytic enzymes required for cell disintegration surrounding the radicle as well as speeding up germination by lengthening the shoots and roots.

Seedling dry weight (g)

Treatment with ammonium molybdate @10^{-3}M revealed the highest mean seedling dry weight, followed by ascorbic acid @ 100ppm, hydropriming, KCl @ 1% & *Pseudomonas fluorescence* @10g/kg, and *Trichoderma viridae* @10g/kg & GA_3 @50ppm (Table 4). All priming treatments outperformed the control by a substantial margin. Seed primed with sodium molybdate enhanced the dry seedling weight in mung bean, according to Umair *et al.*, (2013) and Tiwari *et al.*, (2014) [18] in pigeon pea.

Table 1: Effect of different methods of seed priming on Cowpea seed germination

		Germination percentage			
Treatments	**G_1**	**G_2**	**G_3**	**G_4**	**MEAN**
T_0	59	54	60	52	56
T_1	68	69	72	72	70
T_2	74	75	75	73	74

T_3	80	77	80	81	80
T_4	82	79	85	79	81
T_5	72	68	79	70	72
T_6	76	74	73	76	75
T_7	79	71	75	69	74
T_8	78	77	79	74	77
T_9	73	67	74	67	70
T_{10}	70	70	73	70	71
T_{11}	62	65	67	65	65
T_{12}	68	68	74	68	70
T_{13}	76	72	78	71	74
MEAN	73	70	75	71	
	G		T		GXT
SE.M(±)	0.4		0.7		1.4
CD(0.05)	1.024		1.916		3.831

Note: G = Genotypes, Rairakhol, Sambalpur genotype (G_1), Kantamal, Boudh genotype (G_2), Rupra road, Kalahandi genotype (G_3) and Khariar, Nuapada genotype (G_4), T = Priming treatment, T_0- Control (unprimed), T_1- Hydropriming (Soaking of seeds in de-ionised water for 6 hours), T_2- Osmopriming (KNO_3@ 0.5%, T_3- KNO_3 @ 1%, T_4- KCl @ 1%, T_5- KCl @ 2%), T_6- Biopriming (*Trichoderma viride* @10gram/ kg seed, T_7- *Pseudomonas fluorescence* @10gram/ kg seed), T_8- Hormonal priming (GA_3 @ 50ppm, T_9- GA_3 @ 100ppm, T_{10}-Ascorbic acid @ 100ppm, T_{11}- Ascorbic acid @ 150ppm), T_{12}- Nutripriming (10^{-2}M Ammonium Molybdate and T_{13}- 10^{-3}M Ammonium Molybdate).

Table 2: Effect of different methods of seed priming on Cowpea speed of germination

		Speed of germination			
Treatments	**G_1**	**G_2**	**G_3**	**G_4**	**MEAN**
T_0	10.51	12.24	12.06	11.32	11.53
T_1	14.92	16.27	17.23	19.28	16.93
T_2	20.62	19.83	16.28	15.39	18.03
T_3	20.80	20.99	18.27	15.67	18.93
T_4	21.98	22.25	15.51	16.67	19.10
T_5	15.50	15.03	12.27	11.72	13.38
T_6	18.77	17.87	17.66	12.60	16.72
T_7	18.71	17.33	18.03	16.23	17.58
T_8	21.41	20.66	17.69	14.28	18.51
T_9	15.14	15.37	15.32	11.86	14.42
T_{10}	16.68	16.37	17.56	15.64	16.56
T_{11}	13.71	13.38	15.96	14.39	14.36
T_{12}	15.57	15.22	15.23	15.16	15.30
T_{13}	19.66	18.13	15.11	15.36	17.07
MEAN	17.43	17.21	16.01	14.61	
	G		T		GXT
SE.M(±)	0.085		0.159		0.318
CD(0.05)	0.238		0.445		0.889

Note: G = Genotypes, Rairakhol, Sambalpur genotype (G_1), Kantamal, Boudh genotype (G_2), Rupra road, Kalahandi genotype (G_3) and Khariar, Nuapada genotype (G_4), T = Priming treatment, T_0- Control (unprimed), T_1- Hydropriming (Soaking of seeds in de-ionised water for 6 hours), T_2- Osmopriming (KNO_3@ 0.5%, T_3- KNO_3 @ 1%, T_4- KCl @ 1%, T_5- KCl @ 2%), T_6- Biopriming (*Trichoderma viride* @10gram/ kg seed, T_7- *Pseudomonas fluorescence* @10gram/ kg seed), T_8- Hormonal priming (GA_3 @ 50ppm, T_9- GA_3 @ 100ppm, T_{10}-Ascorbic acid @ 100ppm, T_{11}- Ascorbic acid @ 150ppm), T_{12}- Nutripriming (10^{-2}M Ammonium Molybdate and T_{13}- 10^{-3}M Ammonium Molybdate).

Table 3: Effect of different methods of seed priming on Cowpea seed root length and shoot length (cm)

Treatments	**Root length (cm)**					**Shoot length (cm)**				
	G_1	**G_2**	**G_3**	**G_4**	**MEAN**	**G_1**	**G_2**	**G_3**	**G_4**	**MEAN**
T_0	3.64	3.12	1.53	2.44	2.68	2.40	2.04	1.05	1.11	1.65
T_1	3.83	3.51	2.08	3.06	3.12	1.85	2.95	2.55	2.09	2.36
T_2	4.05	3.78	2.04	2.58	3.11	1.91	2.35	1.56	2.08	1.98
T_3	4.07	4.58	3.63	3.31	3.90	1.85	2.47	2.13	2.51	2.24
T_4	4.15	4.53	3.57	3.22	3.87	2.17	2.62	2.68	1.28	2.19
T_5	4.90	4.38	3.37	2.92	3.89	1.16	2.10	1.53	1.40	1.55
T_6	4.18	4.18	3.33	2.52	3.55	1.97	2.58	1.98	1.32	1.96
T_7	4.34	4.57	2.64	2.57	3.53	2.20	2.05	1.41	1.30	1.74
T_8	4.13	3.62	3.31	3.40	3.62	1.92	2.67	2.13	2.36	2.27
T_9	3.81	4.32	2.61	1.57	3.08	2.40	2.52	2.05	1.21	2.05
T_{10}	3.71	4.40	2.79	2.93	3.46	1.77	2.82	1.62	1.66	1.97

T_{11}	3.72	3.87	2.71	3.41	3.43	1.21	2.80	1.45	1.83	1.82
T_{12}	3.66	3.88	2.68	3.29	3.38	1.85	2.97	1.49	1.48	1.95
T_{13}	4.21	4.61	2.97	3.12	3.73	1.28	2.71	1.22	1.26	1.62
MEAN	4.03	4.10	2.80	2.88	1.85	2.55	1.78	1.64	1.63	
	G		T		GXT	G		T		GXT
SE.M(±)	0.018		0.034		0.069	0.011		0.020		0.040
CD(0.05)	0.051		0.096		0.192	0.030		0.056		0.111

Note: G = Genotypes, Rairakhol, Sambalpur genotype (G_1), Kantamal, Boudh genotype (G_2), Rupra road, Kalahandi genotype (G_3) and Khariar, Nuapada genotype (G_4), T = Priming treatment, T_0- Control (unprimed), T_1- Hydropriming (Soaking of seeds in de-ionised water for 6 hours), T_2- Osmopriming (KNO_3@ 0.5%, T_3- KNO_3 @ 1%, T_4- KCl @ 1%, T_5- KCl @ 2%), T_6- Biopriming (*Trichoderma viride* @10gram/ kg seed, T_7- *Pseudomonas fluorescence* @10gram/ kg seed), T_8- Hormonal priming (GA_3 @ 50ppm, T_9- GA_3 @ 100ppm, T_{10}- Ascorbic acid @ 100ppm, T_{11}- Ascorbic acid @ 150ppm), T_{12}- Nutripriming (10^{-2}M Ammonium Molybdate and T_{13}- 10^{-3}M Ammonium Molybdate).

Seedling Vigour Index-I and II

Seedling Vigour Index-I and II were significantly affected by cowpea genotypes and treatments (Table-5). Kantamal, Boudh genotype had the highest seedling vigour index (105.12), followed by Rairakhol, Sambalpur genotype (94.24), Khariar, Nuapada genotype (83.07) and Rupra road, Kalahandi genotype (82.68). The treatments with KCl @1% had the highest seedling vigour index-I and II followed by KNO_3 @ 1%, GA3 @ 50ppm, *Trichoderma viride* @10g/kg, *Pseudomonas fluorescence* @10g/kg, and ammonium molybdate@10^{-3}M. In comparison to the controls, all seed priming treatments resulted in greater seedling vigour index-I and II. For Seed Vigour Index-II, the interaction impact between the varieties and treatments recorded significant. Kantamal, Boudh genotype with *Trichoderma viride* @10g/kg (139.12) were found to have the highest Seed Vigour Index-II followed by Kantamal, Boudh genotype with GA_3 @ 50ppm (135.52) which are statistically similar for this parameter. The seedling vigour index-I and II as well as varietal responses were significantly affected by different priming treatments. Present results are similarities of the earlier findings of Saeedipour (2013) in cowpea seed, Tiwari *et al.*, (2014) [18] in Pigeon pea and Das *et al*., (2017) in cowpea. Primed seed may have a quicker germination rate, uniform seedling emergence, longer branches and roots as a result of triggering distinct metabolic processes in the seed embryo (Wahid *et al*., 2008 in sunflower). All in all, hydropriming (water), osmopriming (KNO_3 @1%), hormonal priming (GA_3 @50ppm), both biopriming (*Trichoderma viride* & *Pseudomonas fluorescence*), ammonium Molybdate @ 10^{-3}M increased seedling vigour index – I & II and helped in establishing vigorous seedlings.

Table 4: Effect of different methods of seed priming on Cowpea seeds dry weight (g)

Treatments	Dry weight (g)				Mean
	G_1	G_2	G_3	G_4	
T_0	1.12	1.12	0.98	1.09	1.08
T_1	1.55	1.13	1.03	1.18	1.22
T_2	1.24	1.72	1.04	1.24	1.31
T_3	1.19	1.68	0.97	1.10	1.24
T_4	1.27	1.66	0.99	1.26	1.30
T_5	1.24	1.58	1.02	1.21	1.26
T_6	1.15	1.88	0.98	1.15	1.29
T_7	1.44	1.59	0.96	1.21	1.30
T_8	1.29	1.76	0.95	1.17	1.29
T_9	1.28	1.64	1.09	1.11	1.28
T_{10}	1.18	1.82	1.08	1.27	1.34
T_{11}	1.16	1.66	1.11	1.10	1.26
T_{12}	1.32	1.88	1.07	1.31	1.40
T_{13}	1.24	1.46	1.06	1.17	1.23
MEAN	1.26	1.61	1.02	1.18	
SE.M(±)	G		T		GXT
	0.007		0.013		0.025
CD(0.05)	0.019		0.036		0.071

Note: G = Genotypes, Rairakhol, Sambalpur genotype (G_1), Kantamal, Boudh genotype (G_2), Rupra road, Kalahandi genotype (G_3) and Khariar, Nuapada genotype (G_4), T = Priming treatment, T_0- Control (unprimed), T_1- Hydropriming (Soaking of seeds in de-ionised water for 6 hours), T_2- Osmopriming (KNO_3@ 0.5%, T_3- KNO_3 @ 1%, T_4- KCl @ 1%, T_5- KCl @ 2%), T_6- Biopriming (*Trichoderma viride* @10gram/ kg seed, T_7- *Pseudomonas fluorescence* @10gram/ kg seed), T_8- Hormonal priming (GA_3 @ 50ppm, T_9- GA_3 @ 100ppm, T_{10}-Ascorbic acid @ 100ppm, T_{11}- Ascorbic acid @ 150ppm), T_{12}- Nutripriming (10^{-2}M Ammonium Molybdate and T_{13}- 10^{-3}M Ammonium Molybdate).

Table 5: Effect of different methods of seed priming on Cowpea seed vigour index

Treatments	SVI-I				Mean	SVI-II				Mean
	G_1	G_2	G_3	G_4		G_1	G_2	G_3	G_4	
T_0	356.36	278.64	154.80	184.60	243.60	66.08	60.48	58.80	56.68	60.51
T_1	386.24	445.74	333.36	370.80	384.04	105.40	77.97	74.16	84.96	85.62
T_2	441.04	459.75	270.00	340.18	377.74	91.76	129.00	78.00	90.52	97.32
T_3	473.60	542.85	460.80	471.42	487.17	95.20	129.36	77.60	89.10	97.82
T_4	518.24	564.85	531.25	355.50	492.46	104.14	131.14	84.15	99.54	104.74
T_5	436.32	440.64	387.10	302.40	391.62	89.28	107.44	80.58	84.70	90.50
T_6	467.40	500.24	387.63	291.84	411.78	87.40	139.12	71.54	87.40	96.37
T_7	516.66	470.02	303.75	267.03	389.37	113.76	112.89	72.00	83.49	95.54
T_8	471.90	484.33	429.76	426.24	453.06	100.62	135.52	75.05	86.58	99.44
T_9	453.33	458.28	344.84	186.26	360.68	93.44	109.88	80.66	74.37	89.59
T_{10}	383.60	505.40	321.93	321.30	383.06	82.60	127.40	78.84	88.90	94.44
T_{11}	305.66	433.55	278.72	340.60	339.63	71.92	107.90	74.37	71.50	81.42
T_{12}	374.68	465.80	308.58	324.36	368.36	94.24	105.12	82.68	83.07	91.28
T_{13}	417.24	527.04	326.82	310.98	395.52	89.76	127.84	79.18	89.08	96.47
MEAN	428.73	469.80	345.67	320.97		91.83	114.36	76.26	83.56	
	G		T		GXT	G		T		GXT
SE.M(±)	2.063		3.859		7.718	0.482		0.902		1.804
CD(0.05)	5.760		10.776		21.553	1.347		2.519		5.039

Note: G = Genotypes, Rairakhol, Sambalpur genotype (G_1), Kantamal, Boudh genotype (G_2), Rupra road, Kalahandi genotype (G_3) and Khariar, Nuapada genotype (G_4), T = Priming treatment, T_0- Control (unprimed), T_1- Hydropriming (Soaking of seeds in de-ionised water for 6 hours), T_2- Osmopriming (KNO_3@ 0.5%, T_3- KNO_3 @ 1%, T_4- KCl @ 1%, T_5- KCl @ 2%), T_6- Biopriming (*Trichoderma viride* @10gram/ kg seed, T_7- *Pseudomonas fluorescence* @10gram/ kg seed), T_8- Hormonal priming (GA_3 @ 50ppm, T_9- GA_3 @ 100ppm, T_{10}-Ascorbic acid @ 100ppm, T_{11}- Ascorbic acid @ 150ppm), T_{12}- Nutripriming (10^{-2}M Ammonium Molybdate and T_{13}- 10^{-3}M Ammonium Molybdate).

Conclusion

With varied priming methods, Kantamal, Boudh collected genotypes performed best in terms of germination and seedling development followed by Rupra road, Kalahandi genotype and Rairakhol, sambalpur genotype of farmer saved seed. When primed with KCl @ 1% for cowpea seed, the best performing Kantamal, Boudh genotype resulted in the best combination. Seed priming treatments with KCl @ 1%, KNO_3 @ 1%, and *Trichoderma viridae* @ 10g/kg were found to be superior to seed germination percentages that met the minimum seed certification standards specified for the cowpea seed as well as treatments with GA_3 @ 50ppm (hormonal priming) and ammonium molybdate @ 10^{-3}M (nutri-priming).

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