Influence of Seed Priming on Germination and Seedling Growth of *Cicer arietinum L*.

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II. MATERIALS AND METHODS

Abstract- In order to study effects of seed priming with MgSO₄, Salicyclic Acid and Jasmonic acid on the response of two Chick pea varieties viz. Pusa 362 (V_1) and Avrodhi (V_2), an experiment was designed keeping non primed seeds and hydroprimed seeds as controls. Priming response was evaluated at different time intervals of germination in the laboratory. It was found that germination percentage, dry and fresh weight of plumule, radical and cotyledon, amylase activity, proline, protein, soluble sugar and insoluble sugar contents increased in both osmopriming with MgSO₄ and priming with phytohormones like salicyclic acid and jasmonic acid.

The effect of salicyclic acid in respect of germination percentage showed considerable improvement over hydro priming, jasmonic acid as priming agent was not so prominent in comparison to other treatments when germination percentage is considered but it showed a significant increase in respect of root and shoot length and dry weight of plumule.

Keywords—Seed, Cicer arietinum L.

I. INTRODUCTION

Seed priming is a physiological strategy that involves soaking of seeds in a solution of a specific priming agent followed by drying of seeds that initiates germination related process[12]. This has been recognized as an important technology to obtain good germination, rapid development and improved yields in some field crops[3],[9],[2], [10],[13]. Highly successful results have been obtained using priming technology for many crops such as melon [12].

The effectiveness of the priming with simple salt solution, perhaps, depends both on the osmotic potential and the chemical nature of the salt species used. It is reported that nitrate containing compounds may function more efficiently than other salts as priming agents [11]. It has been found many salts can be used very effectively for priming [8]. Reports are available for the use of auxins [7], gibberllins [1], cytokinin [5] to improve seed germination and seedling growth. It has been found that jasmonic acid and salicylic acid plays important role in the seed germination as they are important cellular regulators involved in seed germination, growth of roots, fruit ripening and senescence [6].

In the present experiment therefore an attempt has been mad to study effects of seed priming with MgSO4, Salicyclic Acid and Jasmonic acid on the response of two Chick pea varieties viz. Pusa 362 (V1) and Avrodhi (V2), an experiment was designed keeping non primed seeds and hydroprimed seeds as controls.

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The present experiment was carried out in the Dept. Plant Physiology, Institute of Agricultural Science, Banaras Hindu University, Varansi, Uttar Pradesh. Chickpea was selected for the present experiment. It is widely cultivated in Uttar Pradesh. The varieties taken namely were Avrodhi and Pusa 362. Both of these varieties belong to the desi type with coloured and thick seed coat.

Present experiment was made for short duration up to 5 days to study the percent germination. The seeds were grown on moist filter paper (moistened with distilled water) placed in large sized petri plates (cm) for each study 3 petri plates were used having 50 seeds in each plate. The germination percentage was recorded from 12, 24, 36, 48, 72, 96 & 120h. Similarly, at the same intervals, the length of radicle or plemule were also recorded along with fresh and dry weight, alpha-amylase activity. Besides, soluble and insoluble sugar contents of cotyledons were estimated in different time period. The experiment was conducted in the presence of diffused light condition at a temperature of about 30 C during March – June 2015. In each case the equidistantly placed seeds on the petri plates were in contact with water. In the present study, two varieties of chick pea were taken and they were treated with various chemicals viz. MgSO4 (T3), salicylic acid (T4) and jasmonic acid (T5) in a concentration that was found optimum for various crops as per the literature obtained.

The seeds were considered to be germinated when the radicle was visible through a scar in the seeds. Following formulae was used for calculation:

Germination percent = no. of seeds germinated/ no. of seeds present in the petriplates $x\ 100$

- a) Radicle length (cm) per seed was measured using the formula (L1 + L2 + L3 + ... + Ln)/N
- b) Plemule length (cm) per seed=(X1 + X2 + X3 + ... + Xn)/N
- c) The plumule and radicle portion were separated by sharp blade and weights of both were taken using electronic balance.
- d) The dry weight of shoot and root sample was taken after keeping them in an oven at 100-1100C for one hour. There after these were placed in oven which was set 60 ± 20 C till constant weight was obtained.
- e) The method of Bernfelt (1955) was used to determine \square -amylase activity.
- f) Assay of the enzyme activity was measured as under: Assay mixture was prepared by 1ml substrate (1% starch

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solution prepared in 0.02M phosphate buffer pH 6.9 and 1ml of enzyme activity. The mixture was incubated at 200C for 5mins. The reaction was stoped by adding 2ml of 3,5- di nitro salicylic acid to the incubation mixture. This mixture was kept after heating 5mins in boiling water bath. The mixture was raised upto 25ml by adding distilled water. The final colour of the solution was yellowish orange. The optical density (OD) was measured in a spectrophotometer unibis at 510nm to get theopticaldensity value.

- g) Soluble and insoluble sugar content in cotyledons were determined by the methods of Dubioset al (1956).
 - h) Proline content was estimated by Bates et al (1973).

III. RESULTS AND DISCUSSION

Nature of treatment was seed priming as elaborated in materials and methods section of the present dissertation. Seed were also primed with distilled water called as hydro primed (T2) and non - primed seeds were treated as control (T1). The critical difference (CD) and standard error (SE) were worked out for each case. The results as represented in table 1 to 8 have been described in the following manner:

A. Seed Germination Percentage

It has been found that the percent of seed germination in chickpea increased with the increasing hours (from $12\ h-120\ h$) in both the varieties (Table 1) and after $120\ h$ no improvement in germination was observed. In case of V1 seeds at $12\ h$ maximum percent of germination was observed in MgSO4 primed seeds (T3) followed by salicylic acid(T4) and jasmonic acid (T5). The control (T1) has shown very poor germination representing $11\ \%$ only but hydro priming represented better performance (18 %) as compared to T1, T2 for V1 variety.

In V2 variety, jasmonic acid showed best performance followed by MgSO4 and salicylic acid, fourth position in this respect was occupied by T2 followed by T1, representing non – primed seeds.

Most of the CD at studied hour was found significant with V2 variety. whereas at 24h, 36h and 48h CD showed non-significant value in respect to V1 variety. MgSO4 is an easily available chemical which can improve the germination percentage of chick pea variety as found in case of salicylic acid. But jasmonic acid has shown poor performer as compared to salicylic acid and not easily available also.

TABLE I
EFFECT OF SEED PRIMING WITH WATER SALTS AND HORMONES ON
GERMINATION PERCENTAGE AT DIFFERENT TIME INTERVALS

Treatment	12	h h	24	4 h	36	ő h	48	h	72	h	96	h	12	0 h
Variety	V ₁	V_2	V ₁	V ₂	V ₁	V ₂	V ₁	V_2	V ₁	V_2	V ₁	V ₂	V_1	V_2
t ₁	11.000	14.000	35.30	31.000	39.000	47.000	45.000	56.000	49.000	62.000	54.000	68.000	63.000	77.000
t ₂	18.000	22.000	37.30	61.000	46.000	66.000	55.000	76.000	61.000	80.000	64.000	84.000	71.000	91.000
t ₃	61.000	59.000	75.30	69.000	82.000	82.000	98.000	90.000	100.000	98.000	100.000	100.000	100.000	100.000
t ₄	49.000	55.000	78.30	62.000	88.000	69.000	97.000	79.000	100.000	91.000	100.000	95.000	100.000	97.000
t ₃	42.000	62.000	70.30	72.000	79.000	76.000	86.000	82.000	94.000	90.000	95.000	93.000	98.000	99.000
CD	1.698	2.685	N/A	5.059	N/A	6.643	N/A	5.202	1.925	3.044	1.663	2.629	1.138	1.799
SE (m)	0.572	0.904	1.077	1.703	1.414	2.236	1.108	1.751	0.648	1.025	0.560	0.885	0.383	0.606
	SE(m)	CD	SE(m)	CD	SE(m)	CD	SE(m)	CD	SE(m)	CD	SE(m)	CD	SE(m)	CD
V	0.572	1.698	1.077	N/A	1.414	N/A	1.108	N/A	0.648	1.925	0.560	1.663	0.383	1.138
T	0.904	2.685	1.703	5.059	2.236	6.643	1.751	5.202	1.025	3.044	0.885	2.629	0.606	1.799
V×T	1.278	3.797	2.408	7.155	3.162	9.394	2.477	7.357	1.449	4.305	1.252	3.718	0.856	2.544

T1 =non primed (control) T2 =Hydroprimed T3= $MgSO_4$ T4 = Salicylic acid T5 = Jasmonicacid, V1 =Pusa362 V2 = Avrodhi



Fig 1: Effect of seed priming at different hours of germination in both the varieties Cicer arietinum

B. Length of root and shoot

Table 2 depicts the shoot and root length of chick pea seeds germinated at 120h. The maximum shoot length was recorded at T3 primed set for both the varieties which was followed by T5 for V1 and T4 for V2 variety. In case of root length T3 showed maximum root length for both the varieties which was followed by T4 and T5. Hydro primed set (T2) also showed better performance for both the traits in comparison to non-primed one.

TABLE II
EFFECT OF SEED PRIMING ON SHOOT AND ROOT LENGTH AT 120H OF
GERMINATION

Treatment	Shoot le	ength	Root ler	igth
Variety	V ₁	V_2	V ₁	V_2
t ₁	2.900	3.850	3.400	1.650
t2	5.850	4.600	8.000	5.450
t ₃	20.400	16.500	19.500	19.000
t ₄	10.550	10.750	15.100	13.650
t ₅	11.650	10.400	12.400	12.450
	SE(m)	CD	SE(m)	CD
v	0.379	N/A	0.327	0.971
T	0.600	1.783	0.517	1.535
VxT	0.849	N/A	0.731	N/A

T1 =non primed (control); T2 =Hydroprimed; T3= MgSO₄; T4 = Salicylic acid; T5 = Jasmonicacid; V1 = Pusa 362; V2= Avrodhi.

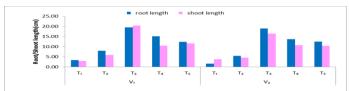


Fig 2: Effect of seed priming with distilled water and different chemicals on shoot and root length at 120h germinated seed

C. Changes in fresh weight

The fresh weight of plumule, radicle and cotyledon for both V1 and V2 are depicted in Table 3.

TABLE III

EFFECT OF SEED PRIMING WITH DISTILLED WATER AND DIFFERENT
CHEMICALS ON FRESH WEIGHT AT 120H GERMINATED SEEDS OF
CHICKPEA(CICER ARIETINUM L.)

	Fresh weight									
Treatment	Plumule		Rad	licle	Cutyledon					
Variety	V_1	V_2	V_1	V_2	V_1	V_2				
t ₁	22.800	11.000	6.150	10.200	316.900	115.900				
t ₂	19.600	15.300	8.750	9.100	405.950	169.050				
t3	19.550	17.650	11.950	12.400	406.350	169.050				
t4	22.000	15.650	13.150	11.850	304.950	185.600				
ts	29 150	17 850	8 950	13 400	265 700	159 200				
	SE(m)	CD	SE(m)	CD	SE(m)	CD				
v	0.576	1.711	0.200	0.593	5.559	16.515				
T	0.910	2.705	0.316	0.938	8.790	26.113				
V×T	1.238	3.825	0.447	1.327	12.431	36.929				

T1 =non primed (control); T2 =Hydroprimed; T3= MgSO4; T4 = Salicylic acid; T5 = Jasmonic acid; V1 =Pusa 362; V2 = Avrodh.

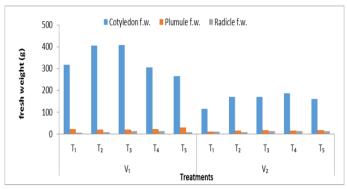


Fig 3: Effect of seed priming with distilled water and different chemicals on fresh weight at 120h germinated seeds of both the varieties i.e. Pusa 362 (V1) and Avrodhi (V2) of chickpea (Cicer arietinum L.)

It is interesting to note that highest increase in fresh weight of plumule was obtained in jasmonic acid treatment (29.15mg) which was followed by T4 for V1 variety and T3 for V2 variety. The cotyledon fresh weight was recorded maximum at T3 for V1 variety and T4 for V2 variety which was followed by T2 set. In V1 variety T5 set and for V2 variety T1 set showed poor performance. The data was found significant for all the studied factors.

D. Influence on dryweight

Table 4 represents effect of priming on dry weight. It is seen that the increase of dry weight of V1 plumule among all treatments was obtained in case of jasmonic acid (15.4 mg) over non primed seeds (12.3 mg). For all other cases of priming the dry weight of plumule decreased from non-primed seeds.

In case of dry weight of radicle in V2 all primed seeds received advantage of increase of dry weight; the highest being MgSO $_4$ primed seeds (302.85mg). In case of dry weight of cotyledon V1 showed significant increase in primed seeds (324.25, 408.015, 215.65 mg respectively for T1, T3 and T4 treatments respectively) except for jasmonic acid which showed a negative result (181.4 mg) in comparison to non-primed seeds (244.35 mg). Similar result was obtained in case of V2 except that jasmonic acid too have positive effect this time (74.0 mg, 77.95 mg, 90.75 mg and 79. 0 mg in T2, T3, T4, T5 treatments) and slightly lower in non-primed seeds (71.45 mg).

TABLE IV

EFFECT OF SEED PRIMING WITH DISTILLED WATER AND DIFFERENT
CHEMICALS ON FRESH WEIGHT AT 120H GERMINATED SEEDS OF CHICKPEA

(CICER ARIETINIM I.)

Dry weight						
Treatment	Plumule	,	Radicle		Cotyledor	1
Variety	V_1	V ₂	V ₁	V ₂	V_1	V ₂
t ₁	12.300	5.150	94.450	152.000	244.350	71.450
t ₂	10.050	8.050	95.750	287.500	324.250	74.000
t ₃	9.500	9.800	77.950	302.850	408.015	77.950
t ₄	10.350	8.250	81.400	247.500	215.650	90.750
t 5	15.400	9.850	75.900	181.400	181.400	79.000
	SE(m)	CD	SE(m)	CD	SE(m)	CD
V	0.332	0.985	2.657	7.892	5.380	15.982
Т	0.524	1.558	4.200	12.479	8.506	25.269
V×T	0.742	2.203	5.940	17.647	12.029	35.736

T1 =non primed (control); T2 =Hydroprimed; T3= MgSO₄; T4 = Salicylic acid; T5 = Jasmonic acid; V1 =Pusa362; V2 = Avrodhi

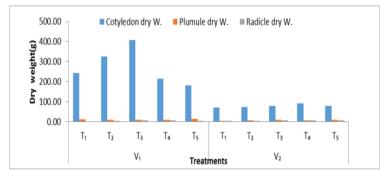


Fig 4: Effect of seed priming on fresh weight at 120h germinated seeds of both the varieties

E. α– *Amylase Activity*

 α -amylase activity of the cotyledons (Table 5) obtained from 72 h to 120 h germinated seeds belonging to two chickpea varieties viz. Pusa 362 (V1) and Avrodhi (V2) raised under five treatments of interest. The T3 treatment showed the highest amylase activity for both the varieties which was

followed by T5 for V1 and T2 for V2 variety. However, at 72h, T2 treatment for V1 variety represented the lowest amylase activity and T4 for V2 variety. As germination hour increased amylase activity was declined. At 120h the maximum amylase activity was recorded at T3 which was followed by T5 and T4. The hydroprimed set also showed better performance in respect to nonprimed set. The data was found statistically significant for variety $V \times T$.

TABLE V
EFFECTS OF SEED PRIMING WITH DISTILLED WATER AND OTHER
CHEMICAL ON A- AMYLASE ACTIVITY (MALTOSE MG G-1FRESH WEIGHT H1) OF CHICK PEA (CICER ARIETINUM L)

Treatment*	α-amylase	activity (Malto	se mg g ⁻¹ Fresh V	Weight h ⁻¹)		
1 reatment	72 h		120 h			
	\mathbf{V}_1	V_2	V_1	V ₂		
t ₁	0.070	0.477	0.023	0.026		
t ₂	0.044	0.288	0.029	0.039		
t ₃	0.334	0.230	0.307	0.235		
t4	0.093	0.135	0.105	0.106		
t ₅	0.247	0.166	0.110	0.128		
	SE(m)	CD	SE(m)	CD		
V	0.038	N/A	0.004	N/A		
T	0.060	N/A	0.007	0.021		
$V \times T$	0.085	0.252	0.010	0.029		

T1 =non primed (control); T2 =Hydroprimed; T3= MgSO₄; T4 = Salicylic acid; T5 = Jasmonic acid; V1 =Pusa362; V2 = Avrodhi.

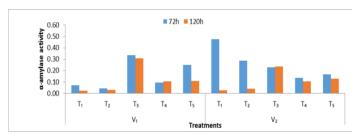


Fig 5: Effects of seed priming with distilled water and other chemical on α– amylase activity (Maltose mg g-1 Fresh Weight h-1) on 72 h and 120h germinated seeds of both the varieties i.e. Pusa 362 (V1) and Avrodhi (V2) of chick pea (Cicero arietinum L).

It is interesting to note that highest increase in fresh weight of plumule was obtained in jasmonic acid treatment (29.15mg) which was followed by T4 for V1 variety and T3 for V2 variety. The cotyledon fresh weight was recorded maximum at T3 for V1 variety and T4 for V2 variety which was followed by T2 primed set. In V1 variety T5 (jasmonic acid) set and for V2 variety T1 (nonprimed) set showed poor performance in respect to other treatments. The data was found significant for all the studied factors.

F. Soluble and Insoluble Sugar

The soluble and insoluble sugar content at 72 h and 120 h germinating cotyledon in dry seeds of chick pea (Table 6) showed very interesting result. In V1 variety maximum soluble sugar content was observed in hydro primed seeds (T2) at 72 h, followed by MgSO4 (T3), Jasmonic acid (T5) and

salicylic acid (T4) primed sets while in case of V2 variety T3 showed best performance followed by T1 and T4 respectively. As germination hour increased the concentration of soluble sugar was increased. At 120 h for V1 variety T2 showed maximum soluble sugar while V2 at T3. The second best result was depicted at T5 followed by T4 and T2 for both the varieties at each studied period. The data was found significant for V \times T.

TABLE VI
EFFECT OF SEED PRIMING WITH DISTILLED WATER AND DIFFERENT
CHEMICAL ON ALCOHOLIC SOLUBLE AND INSOLUBLE SUGAR CONTENT
(MG G-1 DRY WT. L-1) AT 120H GERMINATING SEED OF CHICKPEA
(CICER ARIETUNUML.)

Treatment*	Soluble	sugar conten	ıt (mg g ⁻¹ dr	y wt. l ⁻¹)	Insoluble sugar content (mg g ⁻¹ dry wt. l ⁻¹)				
Treatment	72 h		120 h	120 h		72 h			
	V_1	V ₂	V_1	V_2	V_1	V ₂	V_1	V ₂	
t ₁	0.011	0.014	0.078	0.061	0.030	0.050	0.025	0.007	
t 2	0.017	0.020	0.350	0.046	0.043	0.012	0.010	0.010	
t3	0.090	0.032	0.144	0.090	0.094	0.087	0.064	0.047	
t4	0.023	0.022	0.087	0.050	0.067	0.061	0.048	0.013	
t 5	0.051	0.015	0.122	0.039	0.050	0.029	0.036	0.027	
	CD	SE(m)	CD	SE(m)	CD	SE(m)	CD	SE(m)	
V	0.070	0.024	0.003	0.001	0.008	0.003	N/A	0.005	
T	N/A	0.037	0.005	0.002	0.012	0.004	0.022	0.007	
$\mathbf{V} \times \mathbf{T}$	N/A	0.053	0.007	0.002	0.017	0.006	N/A	0.011	

T1 =non primed (control); T2 =Hydroprimed; T3= MgSO₄; T4 = Salicylic acid; T5 = Jasmonicacid; V1 =Pusa362; V2 = Avrodhi.

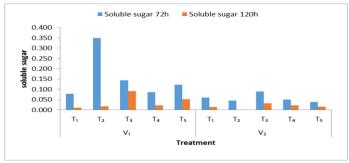


Fig 6 (a): Effect of seed priming with distilled water and different chemical on alcoholic soluble content (mg g-1 dry wt.) at 120h germinating seed of both the varieties i.e. Pusa 362 (V1) and Avrodhi (V2) of chickpea (Cicer arietinum L.)

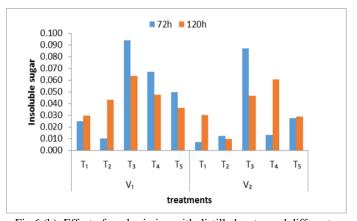


Fig 6 (b): Effect of seed priming with distilled water and different chemical on alcoholic insoluble content (mg g-1 dry wt.) at 120h germinating seed of both the varieties i.e. Pusa 362 (V1) and Avrodhi (V2) of chickpea (Cicer arietinum L.)

G. Proline Content (Mg G-1 Dry Weight)

Table 7 represent the proline content of dry cotyledon of 120 h old germinated seeds of chick pea plants of two different varieties, raised from non-primed (control), distilled water primed, MgSO4 primed, jasmonic acid primed and salicylic acid primed seeds. It has been observed that in both the varieties MgSO4 primed sets (T3) showed the best result which was followed by (T5) and (T4) for V1 and T4 and T5 for V2 variety. In case of V1 variety hydro-primed set showed better performance in comparison to nonprime while in V2 variety non-primed was better. The data was found statistically significant for each studied factors.

TABLE VII

EFFECT OF SEED PRIMING WITH DISTILLED WATER AND DIFFERENT
CHEMICAL ON PROLINE CONTENT (MG G-1 DRY WT./L) CONTENT OF DRY
COTYLEDON COLLECTED FROM 120H GERMINATED CHICK PEA (CICER
ARIETINUM L.)

Treatment	Proline content (mg g	;- ¹ dry wt.)
	V_1	V ₂
t ₁	0.015	0.130
t ₂	0.208	0.083
t ₃	0.899	0.691
t4	0.491	0.541
t ₅	0.788	0.468
CD	0.053	0.084
SE (m)	0.018	0.028
	SE(m)	CD
v	0.018	0.053
T	0.028	0.084
$V \times T$	0.040	0.119

T1 =non primed (control); T2 =Hydroprimed; T3= MgSO₄; T4 = Salicylic acid; T5 = Jasmonic acid; V1 =Pusa362; V2 = Avrodhi.

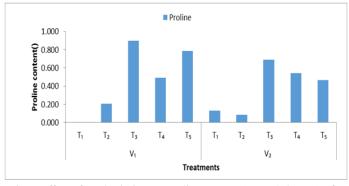


Fig 7: Effect of seed priming on Proline content (mg g-1 dry wt.) of dry cotyledon collected from 120h germinated seeds of both the varieties

H. Protein Content

Table 8 represents the protein content of dry seedling in 120 h old germinated seeds of two chick pea varieties, raised from non-primed (control), distilled water, MgSO₄, jasmonic acid and salicylic acid primed seeds. It has been observed that MgSO₄ primed sets showed best result for both the varieties which was followed by T4 and T5 in V1 while T5 and T4 for V2 variety. The hydroprimed set was also showed better performance in respect to nonprime one for both the varieties. The data was found statistically significant for all studied factors.

TABLE VIII
EFFECT OF SEED PRIMING ON PROTEIN CONTENT OF DRY PLUMULE AND
RADICLE COLLECTED FROM 120HR GERMINATED SEEDS

Treatment	Protein content (mg g-1 dry wt.)						
	\mathbf{V}_1	V_2					
t 1	787.714	755.546					
t ₂	911. 791	923.145					
t 3	1487.845	1300.513					
t4	1297.810	1080.111					
t ₅	1186.768	1182.923					
CD	23.599	7.944					
SE (m)	37.313	12.560					
	SE(m)	CD					
V	7.944	23.599					
T	12.560	37.313					
$V \times T$	17.763	62.769					

T1 =non primed (control); T2 =Hydroprimed; T3= MgSO4; T4 = Salicylic acid; T5 = Jasmonicacid; V1 =Pusa362; V2 = Avrodhi.

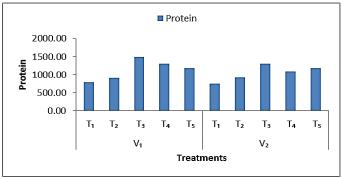


Fig 8: Effect of seed priming on protein content (mg g-1 dry wt.) of dry plumule and radicle collected from 120h germinated seeds of both the varieties

IV. CONCLUSION

In laboratory condition, the germination percentage of both the varities viz. Pusa 362 and Avrodhi of chick pea was increased up to 100% with MgSO₄. The effectiveness of salicyclic acid was found to be more than osmopriming and hydropriming in both the varities in respect of fresh weight (Table 3) and dry weight (Table 4).

On the basis of the result it can be concluded that seed priming would improve stand establishment of chick pea (Cicer arietinum L.), and enable it to compete more successfully with other weed species. Further the general vigour of plant will also increase because of the increase of proline content, protein content and sugar content etc,

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