



Seed quality enhancement of oat (*Avena sativa* L.) varieties through priming

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Abstract

Seed priming through modulating water uptake and subsequent dehydration enhances the germination and field emergence. It helps in imparting the initial vigour to the seedlings which is essential for better field performance. Oat is an important *rabi* fodder cereal and is grown under varying soil/ climate conditions.. In this study, we standardized the priming method and duration for oat seeds. Of the two methods tested for 0-30 h soaking, direct soaking method for 6 h duration was found to be the most suitable for oat seed priming. Four priming treatments viz., hydro-priming, osmo-priming with KNO_3 , hormone priming with GA_3 and vitamin priming with two different doses of Ascorbic acid along with un-primed seeds were studied for their effect on seed quality parameters. The results indicated that priming as such improved the germination, seedling length, dry weight and vigour measured in terms of vigour index-I, II and speed of germination. Among the priming treatments, GA_3 followed by Ascorbic acid resulted in better germination and vigour.

Keywords: Ascorbic acid, Fodder, GA_3 , Hydro priming, Potassium nitrate, Seed priming

Abbreviations: **G:** Grams; **GA_3 :** Gibberellic acid; **H:** Hours; **ISTA:** International seed testing association; **KNO_3 :** Potassium nitrate; **mM:** Milli molar; **P:** Probability; **ppm:** Parts per million; **RH:** Relative humidity

Introduction

Seed germination involves transition of dry seeds from quiescent state to active growing state through imbibition (Van der Geest, 2002). Rehydration results in onset of respiratory activity, membrane repair, metabolic activities including repair and synthesis of macromolecules, synthesis of proteins and cell elongation (Nonogaki *et al.*, 2010). The water uptake by dry mature seeds can be divided into three distinct phases culminating with the radicle protrusion (Bewley and Black, 1994). Seed priming

modulates the water uptake and creates a temporary halt in the process of water uptake thereby ending the process before radicle emergence. This pre-sowing strategy of partial imbibition helps in germination synchrony and improvement of seed performance (Bradford, 1986). By initiating the germination processes it helps in germination enhancement and field emergence particularly under abiotic stress conditions (McDonald, 2000).

Various priming treatments were tested for enhancing the rate of germination, uniformity and plant performance (Bradford, 1986). The methods of priming can be divided into two types based on water uptake viz., un-controlled and controlled priming (Taylor *et al.*, 1998). The simplest method of priming is hydro-priming where uncontrolled hydration occurs. In controlled priming (solid matrix / osmo priming) the hydration will be in an ordered manner. Based on the type of chemical used to improve the seed viability and vigour different types of priming were reported. Seed priming treatments have enhanced germination and improved field establishment in cereals and millets viz., rice, sorghum and maize under stress conditions (Akman, 2009).

Oat is one of the main cereal fodder crop grown in the winter season in the northern, western and central India and is extending even to the eastern region of the country. It is grown worldwide and is one of the oldest cereal crops under cultivation. Oat is a heavy yielder with 45- 55 ton of green fodder per hectare with about 10-12% protein and 30-35% dry matter content. Very few studies were conducted on oat seed priming and were confined to studying the effect of priming on germination percentage (Shafi *et al.*, 2006). In the present study the suitable priming treatment methodology for oat was developed and the effect of various priming treatments on oat seed germination, seedling length, dry weight and vigour index were studied in two varieties differing in their seed characters to find out the suitable priming technique across varieties.

Materials and Methods

Plant materials: Seeds of oat (*Avena sativa*) var. JHO 99-2 and JHO 822 produced during *rabi* 2012-13 were used in different priming experiments. The moisture content of the seeds was estimated using standard hot air oven method of ISTA (2011). Initially the priming technology was standardized for oat variety JHO 99-2 using hydro priming technique. Afterwards the same conditions were employed for all other seed priming treatments in oat varieties.

Seed characterization: The seed parameters viz., seed length, seed width, area, perimeter length, length width ratio and circularity were characterized for the two oat varieties under study using the digital Vernier callipers and by using seed image analysis software, Smart Grain 1.1 as per the methodology of Tanabata *et al.* (2012). The test weight was estimated based on 8 replications of 100 seeds as per ISTA (2011). The seed to husk ratio was estimated by removing husk from 20 seeds in three replications and calculated on weight basis. The moisture content was estimated using standard hot air oven method as per ISTA (2011).

Seed germination: Germination was tested in four replicates of 50 seeds each, following between paper method at 20°C and 80% RH (ISTA, 2011) in the germinator under continuous light condition. The germinated seeds were evaluated and grouped into normal seedlings, abnormal seedlings and dead seeds after ten days. The germination percentage was calculated as the number of normal seedlings out of total seeds kept for germination.

Germination % = (Number of normal seedlings / Total number of seeds) X 100

Seedling length: Seedling length was estimated in three replications following the standard method (Gupta, 1993). Ten normal seedlings were picked randomly from the germinated seeds from each replication. The shoot and root length were measured from the collar region, where shoot joins with root. The seedling length is calculated as the sum of shoot and root length. Subsequently the mean was used for further analysis.

Seedling dry weight: It was estimated in three replications following the standard method (Gupta, 1993). Ten normal seedlings were picked randomly from the germinated seeds from each replication. The seedlings were wrapped in butter paper and kept in oven at 100°C for 24 h. The

dry weight was recorded after cooling in a desiccator containing silica gel. Subsequently the mean was used for further analysis.

Seed Vigour Index (I & II): Quantitative measurement of seed vigour was done by using seed vigour index I and II. The seed vigour index I and II were calculated based on germination (%) and seedling length (cm) and dry weight (g) using the following formula,

Seed Vigour Index I = Germination % x Seedling length (cm) (Abdul. Baki and Anderson, 1973)

Seed Vigour Index II = Germination % x Seedling dry weight (g) (Perry, 1978)

Speed of germination: The earliness in germination is a measure of vigour. It is measured as speed of germination (Bradbeer, 1988). The daily germination count was performed till the end of germination i.e., 10 days. The seeds whose radicle is equal to the size of seed was considered as germinated. After 10 days the speed of germination was calculated using the formula,

Speed of germination = $(N_1 \times 1) + (N_2 \cdot N_1) \times 1/2 + (N_3 \cdot N_2) \times 1/3 + \dots + (N_n \cdot N_{n-1}) \times 1/n$

Where, N_1 , N_2 , N_3 , N_n-1 , N_n : Proportion of germinated seeds observed at first, second, third \dots (n - 1), (n) days

Standardization of seed priming: Priming technique was standardized by employing hydro-priming in oat variety JHO 99-2. On 50 g of seeds hydro-priming was employed both by direct soaking in distilled water and by placing the seeds between the moistened germination towels at ambient laboratory conditions. The duration of treatment was 2, 4, 6, 24, 26, 28 and 30 h. The entire procedure was replicated twice. The hydro-primed seeds were surface dried with absorbent paper and allowed to dry back to their original moisture content under shade in ambient conditions for 48 h. The germination percentage, seedling length, seedling weight, speed of germination and vigour Index I and II were estimated in the primed seeds.

Seed priming treatments: Various priming treatments viz., vitamin-priming, hormone-priming and osmo-priming were done apart from hydro-priming and their effect was studied on seed quality enhancement in comparison with control as following, T_1 : Ascorbic acid (100 ppm), T_2 : Ascorbic acid (50 ppm), T_3 : GA_3 (100 ppm), T_4 : GA_3 (50 ppm), T_5 : Hydro priming, T_6 : KNO_3 (100 ppm), T_7 : KNO_3 (50 ppm), T_8 : con

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-trol (Un-primed). Vitamin-priming was done using Ascorbic acid solution. Whereas in hormone priming GA_3 solution and in osmo-priming KNO_3 solution were used in place of water. All these priming treatments were imposed by directly soaking 25 g of seed material for 6 h at 25°C. Afterwards the seeds were washed with running tap water and surface dried using blotting paper. Further, the seeds were dried back to their original moisture content under shade for 48 h in ambient conditions. The germination percentage, seedling length, speed of germination and vigour indices of primed seeds were tested along with the fresh un-primed seeds as per the standard procedures.

Data analysis: The data was analysed using Analysis for Variance (ANOVA) using single and multi-factor completely randomized block design for laboratory experiment as per Snedecor and Cochran (1994), using statistical software SAS 9.2. Further multiple comparisons of means were done based on Duncun's Multiple Range Test (DMRT).

Results and Discussion

Seed characterization: The two oat varieties, JHO 822 and JHO 99-2 have shown distinct differences in their seed characters. Between the two, JHO 99-2 possess bigger seeds containing 57 percent more area than JHO 822. The perimeter length and circularity of the seed are more ($p < 0.05$) in JHO 99-2 but the length/width ratio and seed to husk ratio of both the varieties are statistically at par (Table 1). The higher moisture content of JHO 822 under similar conditions of storage can be attributed to its smaller size. Test weight is one of important yield character and influences the number of seeds per unit weight. The test weight of JHO 99-2 is more than JHO 822 as it contains bigger seeds (with more length and width). The higher test weight also infers that one kg of JHO 99-2 contains 26,939 seeds whereas in case of JHO 822 it was only 25,201 seeds.

Standardization of hydro-priming: The duration and method of priming differs from species to species. Several reports were there with varying duration of priming treatment for different species (Basra, 1995). In the present study, the duration and method of priming treatment were standardized in oat seeds based on hydro-priming and applied for various other priming techniques. The standardization was done in JHO 99-2 by differential soaking both by direct and between the paper methods. The best method was identified on the basis of seed viability and vigour parameters.

The germination percentage was studied at different time intervals of soaking (0 to 30 h). Between the paper method of soaking has resulted in gradual increase in germination percentage except at 30 h of soaking. Up to four hours of soaking, no significant increase in germination was observed. But from six hours onwards, the germination was significantly superior to un-primed seed with nearly 5% increase (Table 2). Further, after six hours no significant ($p < 0.05$) difference was observed until 24 hours of soaking, where slight decline in germination was noticed. In case of direct soaking method, all the soaking durations resulted in increase in germination percentage as compared to control (86.5%) with maximum germination at 6 h of soaking (92%) (Table 2). No significant difference was observed between 0 h (86.5%) and 2 h (87.5%) of soaking. Similar to between the papers soaking method, after 24 h of soaking slight decrease in germination was observed. Priming not only synchronizes but enhances the germination (Bradford, 1986). Similar results of increase in germination were observed in several crops by previous workers (review by Farooq *et al.*, 2012). The germination studies demonstrated that 6 h of direct soaking resulted in higher germination percentage than between the paper method of soaking. The presence of outer coverings (husk) which reduces the speed of water uptake might be the reason for effective imbibition during direct soaking than between the paper method, where longer durations results in radicle protrusion thereby making the priming ineffective.

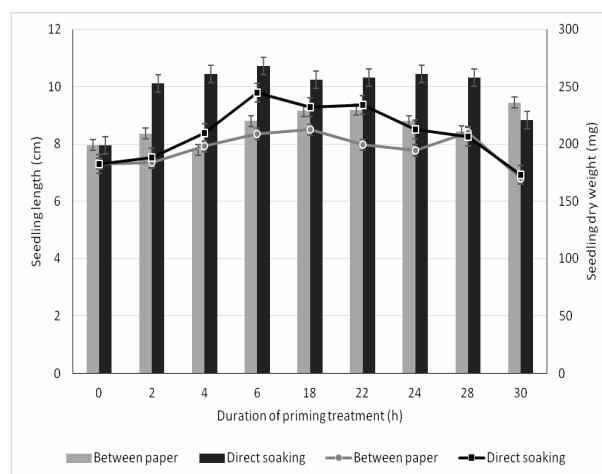


Fig.1. Effect of hydro-priming on seedling length and dry weight in oat variety JHO 99-2

Table 1. Seed characters of oat varieties under study

Variety	SWt (g)	SHR	SW (mm)	SL (mm)	MC (%)	AS (mm ²)	PL (mm)	LWR	Ci
JHO 822	37.12	2.17	2.52	9.63	10.38	18.62	22.00	3.81	0.48
JHO 99-2	39.68	2.31	3.26	11.77	10.03	29.21	26.76	3.62	0.51
Pr > F	0.0002	0.09	<0.0001	<0.0001	0.002	<0.0001	<0.0001	0.06	0.008
CV%	0.61	3.37	6.93	8.76	0.30	11.73	7.80	8.44	6.64

SWt: 1000 seed weight, SHR: Seed to Husk ratio, SW: Seed width, SL: Seed length, MC: Moisture content, AS: Area size, PL: Perimeter length, LWR: Length width ratio Ci: Circularity.

Table 2. Standardization of Hydro-priming in oat seeds (var. 99-2) by between the papers and direct soaking methods

Time (h)	G%		VI-I		VI-II		SG	
	BP	DS	BP	DS	BP	DS	BP	DS
0	86.5 (68.45 ^{cd})	86.5 (68.44 ^f)	632.24 ^{cd}	632.24 ^d	17.25	17.25 ^c	17.78 ^c	17.78 ^d
2	86.5 (68.45 ^{cd})	87.5 (69.29 ^{ef})	634.84 ^{bcd}	659.35 ^d	18.11	22.14 ^{ab}	18.60 ^c	19.77 ^{bc}
4	88.0 (69.73 ^{bc})	89.0 (70.63 ^{de})	697.40 ^{abcd}	746.26 ^c	17.16	23.23 ^{ab}	19.58 ^b	20.21 ^{bc}
6	90.5 (72.05 ^a)	92.0 (73.57 ^a)	757.11 ^{ab}	900.68 ^a	19.91	24.65 ^a	20.51 ^a	22.41 ^a
18	91.5 (73.06 ^a)	91.5 (73.05 ^{ab})	777.77 ^a	850.57 ^{ab}	20.94	23.42 ^{ab}	20.53 ^a	22.04 ^a
22	91.0 (72.57 ^a)	90.5 (72.05 ^{abcd})	725.45 ^{abc}	847.92 ^{ab}	20.91	23.39 ^{ab}	19.88 ^{ab}	20.54 ^b
24	90.5 (72.05 ^a)	91.0 (72.57 ^{abc})	703.89 ^{abc}	774.18 ^{bc}	19.91	23.80 ^{ab}	20.52 ^a	20.36 ^{bc}
28	88.5 (70.18 ^b)	90.0 (71.56 ^{bcd})	745.20 ^{abc}	742.50 ^c	18.67	23.22 ^{ab}	19.93 ^{ab}	19.57 ^c
30	85.0 (67.21 ^d)	89.5 (71.09 ^{cd})	578.85 ^d	620.37 ^d	20.10	19.79 ^{bc}	19.73 ^{ab}	20.10 ^{bc}
SEM	0.51	0.48	34.96	25.89	0.91	1.22	0.26	0.23
LSD (p<0.05)	1.61	1.54	111.86	82.85	NS	3.91	0.85	0.75
CV%	1.01	0.95	7.11	4.86	6.72	7.74	1.90	1.63

Values in parenthesis are arcsine transformed values; Means sharing same alphabet in column are not significantly different (p<0.05). BP: Between paper method, DS: Direct soaking method, G%: Germination percentage, VI-I: Vigour Index-I, VI-II: Vigour Index-II, SG: Speed of germination.

Seedling length, measured as a total of shoot and root length showed no significant difference at differential soaking periods of between paper method (Fig.1). But in direct soaking method highest seedling length was observed at 6 h soaking (9.79 cm). The 4 h to 24 h soaking periods resulted in higher seedling length than the control while the others are at par with control. The seedling dry weight both under direct soaking and between paper methods showed no significant change at different soaking periods (Fig.1).

The seed vigour estimated to find out the field performance potential of the primed seed showed significant enhancement as compared to control under both methods of soaking. The vigour measured as vigour index-I showed significant difference among the priming treatments (Table 2). The between paper method showed highest vigour index-I (777.7) after 18 hours of soaking but it was statistically at par with 4, 6, 22, 24 and 28 h soaking and

superior to 0, 2 and 30 h of soaking. In case of direct soaking method, maximum vigour index (900.68) was noticed at 6 h soaking period which is at par with 18h and 22 h of soaking periods. The vigour index-II based on seedling dry weight could not establish any improvement with priming treatment in both the methods of soaking as well as for different durations of soaking (Table 2). Similar to vigour index-I, the speed of germination, another vigour parameter, showed higher 18 h of soaking in between paper method and 6 h of soaking in direct method. However, the values of 6 h, and 18 h are at par in both the methods (Table 2).

Differential duration treatments revealed that 6 h of soaking is sufficient to find out the effect of priming. Among the two methods, direct soaking has resulted in enhanced germination and vigour as compared to between paper methods. Thus, 6 h direct soaking in the water/ solution is standardized for oat seed priming.

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Table 3. Estimation of viability, seedling length and dry weight of various priming treatments on oat varieties

Varieties	Treatments								Mean
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	
Germination									
JHO 822	92.7 (74.43)	92.0 (73.59)	95.3 (77.54)	92.0 (73.59)	91.3 (72.88)	93.7 (75.43)	94.3 (76.24)	91.0 (72.56)	92.8 (74.53)
JHO 99-2	97.0 (80.12)	97.7 (81.26)	97.7 (82.83)	97.3 (80.64)	96.0 (78.52)	96.3 (79.51)	96.7 (79.93)	94.0 (75.85)	96.6 (77.18)
Mean	94.8 (77.28)	94.8 (77.42)	96.5 (80.18)	94.7 (77.12)	93.7 (75.70)	95.0 (77.47)	95.5 (78.09)	92.5 (74.21)	
Seedling length									
JHO 822	9.72	10.02	10.02	9.66	9.52	9.53	9.68	9.41	9.69
JHO 99-2	9.44	9.76	9.73	9.44	9.49	9.41	9.51	8.93	9.46
Mean	9.58	9.89	9.88	9.55	9.51	9.47	9.6	9.17	
Seedling dry weight									
JHO 822	0.221 ^{cd}	0.232 ^{bc}	0.252 ^a	0.245 ^{ab}	0.225 ^{cd}	0.220 ^{cd}	0.224 ^{cd}	0.211 ^d	0.229
JHO 99-2	0.251 ^a	0.255 ^a	0.254 ^a	0.249 ^a	0.248 ^a	0.245 ^{ab}	0.247 ^a	0.225 ^{cd}	0.238
Mean	0.236	0.244	0.253	0.247	0.236	0.233	0.236	0.218	

	Germination	Seedling length	Seedling dry weight
LSD (p<0.05) for variety	1.43	0.2	0.004
LSD (p<0.05) for treatment	2.85	0.39	0.009
LSD (p<0.05) for variety X treatment	NS	NS	0.013
CV%	3.14	3.49	3.19

Values in parenthesis are arcsine transformed values; Means sharing same alphabet in column are not significantly different ($p \leq 0.05$). T₁: Ascorbic acid (100 ppm), T₂: Ascorbic acid (50 ppm), T₃: GA₃ (100 ppm), T₄: GA₃ (50 ppm), T₅: Hydro priming, T₆: KNO₃ (100 ppm), T₇: KNO₃ (50 ppm), T₈: control (Un-primed).

Table 4. Estimation of seed vigour traits of various primed oat varieties

Varieties	Treatments								Mean
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	
Vigour Index-I									
JHO 822	901.69	921.58	955.2	889.05	869.77	892.49	913.04	856.31	899.89
JHO 99-2	915.41	952.9	950.75	918.96	910.72	905.87	919.63	839.42	914.21
Mean	908.55	937.24	952.98	904.01	890.25	899.18	916.33	847.87	
Vigour Index-II									
JHO 822	20.51 ^{de}	21.37 ^{cd}	24.02 ^a	22.51 ^{bc}	20.52 ^{de}	20.64 ^{de}	21.13 ^{cd}	19.20 ^e	21.24
JHO 99-2	24.37 ^a	24.91 ^a	24.85 ^a	24.27 ^a	23.78 ^{ab}	23.57 ^{ab}	23.87 ^{ab}	21.15 ^{cd}	23.85
Mean	22.44	23.14	24.44	23.39	22.15	22.11	22.50	20.18	
Seed of germination									
JHO 822	19.21	19.06	19.47	19.31	19.07	19.25	19.39	18.34	19.14
JHO 99-2	20.89	21.2	21.02	20.87	20.7	20.72	20.87	19.65	20.74
Mean	20.05	20.13	20.25	20.09	19.88	19.98	20.13	18.99	

	Vigour Index-I	Vigour Index-II	Speed of germination
LSD (p<0.05) for variety	NS	0.48	0.14
LSD (p<0.05) for treatment	41.60	0.96	0.28
LSD (p<0.05) for variety X treatment	NS	1.36	NS
CV%	3.90	3.63	1.18

Means sharing same alphabet in column are not significantly different ($p \leq 0.05$).

T₁: Ascorbic acid (100 ppm), T₂: Ascorbic acid (50 ppm), T₃: GA₃ (100 ppm), T₄: GA₃ (50 ppm), T₅: Hydro priming, T₆: KNO₃ (100 ppm), T₇: KNO₃ (50 ppm), T₈: control (Un-primed)

Effect of priming treatments: Priming showed a positive effect on germination and other quality traits. Overall enhancement of germination, seedling length and dry weight was recorded (Table 3). In the present study, both the varieties showed variability ($p < 0.05$) with respect to viability and seedling characters. Of the two varieties JHO 99-2 showed higher ($p < 0.05$) values for germination (96.6%) and seedling dry weight (0.238 g) than JHO 822 (92.8% & 0.229 g). Similar kind of varietal variance during priming was recorded in other crops (Raj *et al.*, 2013). The variation in seed characters *viz.*, size, area, perimeter *etc.* might have resulted in the variation of response of varieties. Maximum germination was recorded in GA_3 (100 ppm) treatment in both the varieties (Table 3). Increase in seedling length (7.7%) and dry weight (16%) was also noticed compared to control. Similar results were obtained by treating the wheat seeds with 20 ppm GA_3 (Khan *et al.*, 2011). No interaction effect of varieties x treatments was found in germination and seedling length. In case of seedling dry weight, except control and GA_3 treatment, JHO 99-2 responded better than JHO 822.

The seed vigour measured in terms of vigour index and speed of germination indicated the superiority of primed seed over control in both the varieties (Table 4). The variation in seed vigour between varieties was observed through vigour index-II and speed of germination which showed 12.3% and 8.3% higher values ($p < 0.05$) in JHO 99-2 than JHO 822. Varietal mean indicated that priming treatment with 100 ppm GA_3 has resulted in highest vigour index-I and II (952.98 & 24.44) among the treatments. Similar results were noticed in speed of germination where 6.6% increase over control was observed. GA_3 is a key hormone in regulating the germination mechanism by activating several enzyme systems in the early phases and helps for a fast radicle protrusion and hypocotyl elongation (Maske *et al.*, 1997). Seed priming with GA_3 enhances the conversion of starch to sugars with increased amylase activity (Afzal *et al.*, 2008) resulting in augmented energy availability for faster germination with improved vigour. The increase in seed germination and vigour parameters due to GA_3 as observed in present study was noticed by several earlier workers in various cereal crops (Afzal *et al.*, 2008; Akman, 2009; Ghobadi *et al.*, 2012). No interaction effect of varieties x treatments was observed in vigour index-I and speed of germination, but in vigour index-II which is based on seedling dry weight, JHO 99-2 showed better positive response with priming treatments than JHO 822. After GA_3 the Ascorbic acid treatment (50 ppm) was found to be the second best priming treatment in oat seeds with 10-15 percent vigour increm-

-ent (Table 4) than control. Similar kind of increased vigour was observed in wheat seeds treated with 0.6 mM ascorbic acid (Al-Hakimi and Hamada, 2001). Enhanced replication in root tips of hormonal and vitamin primed seeds (Shakirova *et al.*, 2003) might be the reason for augmented vigour index and speed of germination. The hydro-priming and osmo-priming (KNO_3) treatments also showed enhanced germination and vigour as compared to unprimed seed (Table 4). Priming has a positive effect on germination and vigour which might be due to increased metabolic activities (Soon *et al.*, 2000), activation of reserve mobilization enzymes, synthesis of macromolecules, repairing of DNA and by enhanced ATP content and energy charge (Varier *et al.*, 2010).

Conclusion

In conclusion, the present findings indicate that oat seeds respond well to direct soaking for 6h under ambient conditions ($35 \pm 2^\circ C$) resulting in better germination (92%) as compared to control (86.5%) as well as other priming durations. Further, the vigour studies strengthened this finding with increased seedling length and dry weight as well as speed of germination. Among the priming treatments GA_3 (100 ppm), followed by Ascorbic acid (50 ppm) have resulted in better viability and vigour as compared to un-primed seed in both the oat varieties with varying seed characters.

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