

Effect of Inositol on Post Harvest Germination of Groundnut (*Arachis hypogaea* L.) Genotypes under Stress

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ABSTRACT

A field experiment was conducted at the Main Agricultural Research Station, University of Agricultural Sciences, Dharwad during Kharif. The experiment consisted of two treatments, foliar application of inositol @ 100 ppm at 65 DAS (during pod and seed development) in 10 genotypes of groundnut (TAG 24, JL 24, R-2001-3, K-07, GPBD 4, GPBD 5, K-134, TKG-19A, Girnar 1, and GPBD 6) and another set of genotypes maintained without application as control. The experiment was laid out in factorial design with 20 treatment combinations in three replications. The data on post harvest germination of groundnut seeds after 48 hour indicated that seed obtained from inositol sprayed groundnut recorded 10 per cent higher germination compared to control. Higher germination could be attributed to increased availability of sugar from the accumulation of phosphorus during seed. Seed harvested from inositol treated plant showed 10 per cent higher germination under PEG stress condition of -10 bar confirming the role of inositol in increasing the moisture stress resistance. Irrespective of PEG- stress or under control (well water condition) at 30 days after storage the seeds obtained from inositol treatment plants performed better. Thus, the quality of the seeds of inositol treated plant during pod filling could be better and has been shown here in the present investigation in the form of higher germination and inositol acts as osmoprotectant.

Key words: Inositol, Groundnut, Stress, Phytate

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important food legume and oilseeds crop. Groundnut is a unique crop, combining the attributes of both oilseed and legume crops in the farming system of Indian agriculture. It is a valuable crop planted in the dry area of Asia, Africa, Central and South America, Australia and Caribbean in view of its economic, food and nutritional value. It is a primary source of

edible oil and has a high oil (44 to 55%) and protein content (25%) and is also a valuable source of vitamin E, K, and B. The chemical formula of *myo*-inositol (inositol) is $C_6H_{12}O_6$. *Myo*-inositol is synthesized from glucose-6-phosphate (G-6-P) in two steps. First, G-6-P is isomerised by ISYNA1 to *myo*-inositol 1-phosphate, which is then dephosphorylated by IMPase 1 to give free *myo*-inositol⁶.

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Phytate (phytic acid) is the most abundant *myo*-inositol phosphate (hexakisphosphate) in plant cells. Phytate serves as a storage form of *myo*-inositol, phosphate and mineral nutrients for utilization during seed germination and seedling growth. It is deposited within single-membrane storage organelles referred to as protein bodies and is usually present as mixed complexes with minerals (K^+ , Mg^{2+} , Ca^{2+} , Fe^{2+} , Zn^{2+}) known as phytin^{7,8}. Gupta *et al.*³ also acknowledge that phytate play important role in germination of seed.

The phytate is utilised as a source of inorganic phosphate during seed germination and the inorganic form becomes available for purpose of the growth and development. The liberation of phosphate from phytate occurs by enzyme hydrolysis. Phytase is the currently accepted enzyme, which is responsible for the complete hydrolysis of phytate (Inositol hexaphosphate) into inositol and phosphate. Germination reduces and/or eliminates considerable amounts of phytate from the seed or grain. Disappearance of phytate during germination depends on the phytase activity. A rapid rise in phytase activity was observed commencing after 48h germination of bush bean. Germination reduced the phytic acid content of chickpea and pigeonpea seed by over 60% and soybean by about 40 per cent¹⁰. However, there is little data on the post harvest germination response of groundnut genotypes under stress condition after application of inositol. It is necessary to observe inositol effect on the physiological response like germination is particularly important for successful stand establishment and growth by groundnut genotypes and their interaction with inositol in groundnut plant system. The information on the effect of inositol on seed germination of legumes and oil seed crops is meagre. Therefore the present investigation was aimed to find out effect of inositol on seed germination under normal and stress condition with following objectives:

➤ To find out differential responses of groundnut cultivars to inositol for seed germination at different time interval.

➤ To study the effect of inositol on post harvest germination under stress.

MATERIAL AND METHODS

A field experiment was conducted during *kharif* at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad to assess post harvest germination of the seeds of the groundnut genotypes due to inositol were also made in the laboratory. The soil of experimental site was medium black clay loam soil. Composite soil samples were analyzed from the experimental site for various physical and chemical properties, the details along with employed are presented in Table 1. The groundnut genotypes TAG 24 (V₁), JL 24 (V₂), R-2001-3 (V₃), K-07 (V₄), GPBD 4 (V₅), GPBD 5 (V₆), K-134 (V₇), TKG-19A (V₈), Girnar 1(V₉) and GPBD 6 (V₁₀) were obtained from groundnut breeder AICRP on oilseeds, MARS, UAS Dharwad for the study. The silent features of genotypes are presented in Table 2. Foliar application of inositol (*cis*-1,2,3,5-*trans*-4,6 Cyclohexanehexol) @ 100 ppm concentration as per treatment details was done at 65 days after sowing (DAS).

Land preparation

The experimental field was brought to a fine tilth by once ploughing and two times harrowing after harvest of previous general crop, maize. Experimental plots were levelled with wooden plank to bring the field to a fine tilth.

Sowing of seeds and spacing

Sowing was done in *kharif* on 14th June 2008 by hand dibbling. The seeds were sown at a distance of 20 cm between the row and 10 cm between the plants. Complete care was taken to control the pests and diseases during the crop growth period. The genotypes were harvested as and when the genotype attained physiological maturity.

Fertilizer application and Harvesting

Recommended dose of 15:25:15 kg N: P₂O₅: K₂O per ha was applied. Full dose of N, P₂O₅ and K₂O was applied at 25 DAS. The genotypes were harvested as and when they attained physiological maturity.

Germination under PEG stress condition

Germination of groundnut seeds was taken on percent basis at 7 day and 30 day interval and 90 days interval after harvesting of the crop to study the seed dormancy under storage condition (storage period). Seeds were kept for germination on germination paper in humidity chamber at 25 °C - 28 °C temperature and 95±3 percent relative humidity and also in PEG-6000 solution at -10 bars after 90 days of harvesting.

Statistical analysis

The data was subjected to the analysis of variance following the method of Panse and Sukhatme (1967). The level of significance in “F” and “t” tests was P=0.05. The critical differences (C.D) were calculated whenever “F” test was found significant.

RESULTS

Germination percentage 7 days after harvest (7 DAH)

The total germination recorded at 7 DAH is presented in Table 3. The total germination percentage after 24 hour was significantly higher in inositol (53.17 %) when compared to control (41.90 %). The genotypes also differed significantly. The genotype GPBD 6 (71.50 %) recorded significantly higher germination percentage followed by the genotype K-134 (71.17 %). The lowest germination was recorded in K-07 (28.17%). The interaction effects of genotypes and treatments were also significant. The genotype K-134 recorded significantly higher germination percentage with application of inositol (80.33 %). After 48 hour observation on germination showed inositol (100 ppm) again maintained higher germination percentage and it was higher by 14.73 percent. As regard to genotypes the genotype K-134 (85.67 %) had significantly higher germination per cent followed by TKG-19A (83.33 %). The interaction between treatments and genotypes was significant. The genotype K-134 (85.67 %) recorded higher germination per cent in inositol application after 48 hours after 30 days of storage.

Germination percentage 30 days after harvest (30 DAH)

In general, the germination seven days after harvest and 30 days after harvest remained almost similar (Table 3 and 4). The germination per cent recorded at 30 DAH is presented in Table 30 indicated that germination per cent after 24 hour was significantly higher due to the application of inositol as compared to control. The genotypes also differed significantly. The genotype GPBD 6 recorded significantly higher germination per cent (69.8 %) followed by the genotype K-134 (69.33 %). The lowest germination per cent was recorded in K-07 (27.67 %). The interaction effects of genotypes and treatments were also significant. The genotype GPBD 6 recorded significantly higher germination per cent with application of inositol *i.e.* (79.33 %). After 48 hour of germination showed that inositol again maintain higher germination per cent and it was higher by 15%. As regards to genotypes K-134 had significantly higher germination per cent (84.67 %) followed by TKG-19A (82.00 %). The interaction effect between treatment and genotypes was significant. The genotype R-2001-3 recorded higher germination per cent (86.67 %) in inositol followed by K-134 (86.33 %).

Germination per cent under PEG stress condition

The germinability of kernel obtained from inositol application (100 ppm) was studied under PEG stress condition. The germination was observed after 48 hours of incubation in Table 5. The germination under PEG stress condition was lower compared to non stress condition (32 %). However, the inositol treated seeds performed better under both conditions compared to their respective control (28 %). There was 23 per cent higher germination due to inositol under PEG stress condition. Where as in under water sufficient condition there was 16 per cent increase in germination compared to control. Thus there was improved germination under PEG stress condition and non stress condition due to inositol application (100 ppm). Among the genotypes, genotype

GPBD 4 recorded significantly higher germination followed by GPBD 6 and significantly lower germination was recorded in JL-24 under PEG stress condition. Under non stress condition the genotype TKG-19A recorded the higher germination (78.1 %) followed by the genotype GPBD 6 (77.7 %). The lowest was recorded in the genotype TAG 24. The interaction effect between treatments and genotypes were also significant. Inositol applied GPBD 4 recorded significantly higher germination per cent compared to any of the treatment combinations. Where as the genotype R-2001-3 and TKG-19A which are inositols (100 ppm) treated recorded higher germination compared to control or any other combination. The significantly lowest germination per cent was recorded in the genotype TAG 24 under control (41.3 %).

DISCUSSIONS

Germination is one of the important processes for the crop stand establishment in the field. Successful germination not only dependent on environmental factors but also on the quality of the seed. It is both organic and inorganic contents of the seed that measures the quality. The phytate is one of the important constituents of leguminous seeds comprising of different salts viz. Ca, Mg, P and other micronutrients. The phytate is utilized as a source of inorganic phosphate during seed germination and the inorganic form becomes available for purpose of plant growth and development¹⁰.

The data on post harvest germination of groundnut seeds after 48 hour indicated that seed obtained from inositol sprayed groundnut recorded 10 per cent higher germination compared to control. Higher germination could be attributed to increased availability of sugar from the accumulation of phosphorus during seed.

D-*chiro*-Inositol and *myo*-inositol were found in lower amounts than those of pinitol. These free cyclitols were present in higher concentrations, mainly in embryonic axes, increasing consistently when

germination reached 100% in *C. echinata* seeds under field conditions¹. Analysis of sugars during storage under different temperature conditions showed low levels of glucose and fructose in relation to sucrose in seeds of *C. echinata* that had lost germinability², suggesting that sucrose metabolism could be related to seed viability and the ratio of monosaccharide's to disaccharides is an indication of this process. The decline in vigor of the maize embryo was associated with a marked decline in monosaccharides and in raffinose¹.

Seed harvested from inositol treated plant showed 10 per cent higher germination under PEG stress condition of -10 bar conforming the role of inositol in increasing the moisture stress resistance as has been reported by Ishitani *et al.*⁵. Irrespective of PEG- stress or under control (well water condition) at 30 days after storage the seeds obtained from inositol treatment plants performed better. Thus, the quality of the seeds of inositol treated plant during pod filling could be better and has been shown here in the present investigation in the form of higher germination and inositol acts as osmoprotectant^{4,5}.

The pathway from glucose 6-phosphate (G 6-P) to *myo*-inositol 1-phosphate (Ins 1-P) and *myo*-inositol (Ins) is essential for the synthesis of various metabolites. In the halophyte *Mesembryanthemum crystallinum* (common ice plant), two enzymes, *myo*-inositol O-methyltransferase (IMT1) and ononitol epimerase (OEP1), extend this pathway and lead to the accumulation of methylated inositols, D-ononitol and D-pinitol, which serve as osmoprotectants⁵. Sheveleva *et al.*⁹ reported that transferred cDNA encoding *myo*-inositol O-methyltransferase (IMT1) in to *Nicotiana tabacum* (SR1) had less inhibition of photosynthetic CO₂ fixation during drought and salt stress and concluded that solute accumulation appears to provide better protection under drought by osmotic adjustment.

Table 1: Physical and chemical properties of the soil in the experimental site

SI. No.	Properties	Value obtained	Method employed
I	Physical properties		
A	Particles size analysis		
1	Coarse sand (%)	6.28	International pipette method (Piper, 1966)
2	Fine sand (%)	14.27	
3	Silt (%)	27.52	
4	Clay (%)	51.99	
B	Bulk density (mg/m³)	1.33	Core sample method (Dastane, 1967)
II	Chemical properties		
1	Soil pH (1:2.5 soil water)	7.60	pH meter (Piper, 1966)
2	Electrical conductivity (dS/m)	0.28	Conductivity bridge (Jackson, 1967)
3	Organic carbon (g/kg)	5.20	Walkley and blacks wet oxidation method (Jackson, 1967)
4	Available nitrogen (kg/ha)	221.0	Modified kjeldhal method (Jackson, 1967)
5	Available phosphorus (kg/ha)	32.40	Olsen' method (Jackson, 1967)
6	Available potassium (kg/ha)	318.70	Flame photometer (Jackson, 1967)

Table 2: Description of groundnut genotypes

SI. No.	Genotype	Botanical type	100 seed weight (g)	Oil (%)	Yield (q/ha)	Silent feature
1	TAG 24	Bunch type	37-42 g	40-48 %	32-35	Drought resistant
2	JL 24	Bunch type	26-52 g	41-49 %	24-35	Drought resistant
3	R-2001-3	Bunch type	29-35 g	40-45 %	26-50	Drought resistant
4	K-04	Bunch type	30-45 g	45-48 %	45-60	Drought resistant
5	GPBD 4	Bunch type	30-35 g	48-53 %	40-50	Rust resistant
6	GPBD 5	Bunch type	32-45 g	42-49 %	40-50	Rust resistant
7	K-134	Bunch type	25-30 g	42-48 %	40-55	Drought resistant
8	TKG-19A	Bunch type	40-60 g	36-46 %	35-50	Bold seed
9	Girnar 1	Bunch type	30-55 g	40-45 %	45-60	Drought resistant
10	GPBD 6	Bunch type	25-35 g	42-46 %	35-45	Rust resistant

Table 3: Effect of inositol on germination (%) in groundnut genotypes after 7 of days of harvest

Genotypes	After 24 hour			After 48 hour		
	Treatments		Mean	Treatments		Mean
	T ₁	T ₂		T ₁	T ₂	
TAG 24	25.0 (30.0)*	38.3 (38.2)	31.7 (34.1)	45.7 (42.5)	55.3 (48.0)	50.5 (45.3)
JL 24	57.0 (49.0)	63.0 (52.7)	60.2 (50.9)	66.7 (54.7)	86.0 (68.0)	76.3 (61.4)
R-2001-3	45.7 (42.5)	50.7 (45.4)	48.2 (43.9)	72.0 (58.0)	87.7 (69.4)	79.8 (63.7)
K-07	24.7 (29.8)	31.7 (34.2)	28.2 (32.0)	56.0 (48.4)	70.0 (56.8)	63.0 (52.6)
GPBD 4	45.3 (42.3)	55.3 (48.0)	50.3 (45.2)	66.0 (54.4)	80.7 (63.9)	73.3 (59.1)
GPBD 5	37.3 (37.6)	41.0 (39.8)	39.2 (38.7)	63.3 (52.7)	65.7 (54.1)	64.5 (53.4)
K-134	62.0 (51.9)	80.3 (63.7)	71.2 (57.8)	84.0 (66.4)	87.3 (69.2)	85.7 (67.8)
TKG-19A	24.3 (29.5)	35.0 (36.3)	29.7 (32.9)	79.3 (63.0)	87.3 (69.1)	83.3 (66.1)
Girnar 1	35.7 (36.6)	55.0 (47.8)	45.3 (42.2)	75.7 (60.5)	86.0 (68.0)	80.8 (64.2)
GPBD 6	62.0 (51.9)	81.0 (64.2)	71.5 (58.0)	79.0 (62.7)	83.0 (65.7)	81.0 (64.2)
Mean	41.9 (40.1)	53.2 (47.0)	47.5 (43.6)	68.8 (56.3)	78.9 (63.2)	73.8 (59.8)
For Comparing mean of	S. Em±		CD (5%)	S. Em±		CD (5%)
Treatments	0.3		0.9	0.6		1.7
Genotypes	0.7		2.1	1.3		3.7
Treatments × Genotypes	1.0		3.0	1.8		5.3

T₁ = ControlT₂ = Inositol (100 ppm)

NS = Non significant

* The value in paranthesis indicate arc sine transformed value

Table 4: Effect of inositol on germination (%) in groundnut genotypes after 30 of days of harvest

Genotypes	After 24 hour			After 48 hour		
	Treatments		Mean	Treatments		Mean
	T ₁	T ₂		T ₁	T ₂	
TAG 24	24.7 (29.8)*	37.7 (37.8)	31.2 (33.8)	44.3 (41.7)	54.0 (47.3)	49.2 (44.5)
JL 24	55.3 (48.0)	62.0 (51.9)	58.7 (50.0)	65.7 (54.1)	85.0 (67.2)	75.3 (60.7)
R-2001-3	43.0 (41.0)	49.3 (44.6)	46.2 (42.8)	71.0 (57.4)	86.7 (68.5)	78.8 (63.0)
K-07	24.3 (29.5)	31.0 (33.8)	27.7 (31.7)	55.3 (48.0)	68.7 (55.9)	62.0 (52.0)
GPBD 4	43.7 (41.3)	52.0 (46.1)	47.8 (43.7)	65.0 (53.7)	80.0 (63.4)	72.5 (58.6)
GPBD 5	37.3 (37.6)	39.3 (38.8)	38.3 (38.2)	62.7 (52.3)	64.0 (53.1)	63.3 (52.7)
K-134	60.3 (50.9)	78.3 (62.2)	69.3 (56.6)	83.0 (65.6)	86.3 (68.3)	84.7 (67.0)
TKG-19A	23.0 (28.6)	34.0 (35.6)	28.5 (32.1)	77.7 (61.8)	86.3 (68.3)	82.0 (65.1)
Girnar 1	34.7 (36.0)	53.3 (46.9)	44.0 (41.5)	74.3 (59.6)	85.3 (67.5)	79.8 (63.5)
GPBD 6	60.3 (50.9)	79.3 (63.0)	69.8 (56.9)	77.7 (61.8)	82.3 (65.2)	80.0 (63.5)
Mean	40.7 (39.4)	51.6 (46.0)	46.1 (42.7)	67.7 (55.6)	77.9 (62.5)	72.8 (59.0)
For Comparing mean of	S. Em±		CD (5%)	S. Em±		CD (5%)
Treatments	0.4		1.0	0.6		1.6
Genotypes	0.8		2.3	1.3		3.6
Treatments × Genotypes	1.1		3.3	1.8		5.1

T₁ = ControlT₂ = Inositol (100 ppm)

NS = Non significant

* The value in paranthesis indicate arc sine transformed value

Table 5: Effect of inositol on germination (%) in groundnut genotypes under PEG stress condition

Genotypes	After 48 hour					
	PEG-6000 solution (-10 bar)			Water (0 bar)		
	Treatments		Mean	Treatments		Mean
	T ₁	T ₂		T ₁	T ₂	
TAG 24	31.7 (34.2)*	47.7 (43.6)	39.7 (38.9)	41.3 (40.0)	53.3 (46.9)	47.3 (43.4)
JL 24	11.0 (19.3)	11.3 (19.6)	11.2 (19.5)	64.3 (53.3)	82.0 (64.9)	73.2 (59.1)
R-2001-3	12.0 (20.2)	13.3 (21.3)	12.7 (20.8)	68.7 (55.9)	84.0 (66.4)	76.3 (61.2)
K-07	42.7 (40.8)	52.3 (46.3)	47.5 (43.5)	53.3 (46.9)	66.7 (54.7)	60.0 (50.8)
GPBD 4	53.7 (47.1)	75.3 (60.2)	64.5 (53.6)	63.7 (52.9)	78.3 (62.2)	71.0 (57.6)
GPBD 5	42.7 (40.8)	48.0 (43.8)	45.3 (42.3)	58.0 (49.6)	61.3 (51.5)	59.7 (50.6)
K-134	12.0 (20.2)	20.0 (26.5)	16.0 (23.4)	81.3 (64.4)	83.3 (65.9)	82.3 (65.2)
TKG-19A	11.7 (19.9)	12.0 (20.2)	11.8 (20.1)	73.0 (58.7)	83.3 (65.9)	78.2 (62.3)
Girnar 1	12.7 (20.8)	13.0 (21.1)	12.8 (20.9)	71.0 (57.4)	81.0 (64.1)	76.0 (60.8)
GPBD 6	57.7 (49.4)	60.0 (50.7)	58.8 (50.1)	75.0 (60.0)	80.3 (63.7)	77.7 (61.8)
Mean	28.8 (31.3)	35.3 (35.4)	32.0 (33.3)	65.0 (53.9)	75.4 (60.6)	70.2 (57.3)
For Comparing mean of	S. Em±		CD (5%)	S. Em±		CD (5%)
Treatment	0.2		0.6	0.3		0.8
Genotypes	0.5		1.4	0.64		1.8
Treatment × Genotypes	0.7		2.0	0.91		2.6

T₁ = ControlT₂ = Inositol (100 ppm)

NS = Non significant

* The value in parenthesis indicate arc sine transformed value

CONCLUSION

Seed germination and viability of groundnut seeds are subjected to variations during storage conditions and germination under stress condition. It has been found in the present study that it is possible to increase the germination of groundnut seeds up to 30 days of harvesting and under stress condition by application of inositol. Since seed is an important input in agriculture which determines not only the production but also the productivity, it is essential to maintain the seed quality and germination.

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