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Retention of Seed Storage Potential Using Ascorbic Acid

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Abstract: An investigation was carried out on prolongation of seed vigour of a black gram species by using a selected chemical. Black gram seeds lost viability at a rapid pace under accelerated ageing condition. Pretreatment of black gram (*Vigna mungo* L.) seeds with ascorbic acid for 6 hours (3+3) before accelerated ageing treatment (100% RH and 32±2°C) for different durations (0 to 30 days) slowed down the ageing-induced rapid loss of germination. The chemical also significantly arrested the reduction of protein, insoluble carbohydrate levels as well as activity of catalase enzyme of seed kernels during forced ageing period was ameliorated to a significant extent in the chemical-pretreated seed. Conversely, ageing-induced stimulation of the activity of amylase enzyme was alleviated by the seed pretreating agent. Seed potential was found to be much better in the pretreatments as evidenced from the treatment-induced higher protein and activity of catalase enzyme in spite of adverse storage situation. Results, therefore, pointed out that the ascorbic acid pretreated seeds retained higher seed vigour of black gram species. The promising effects of the experimental chemical on storage potentiation of the seed is apparent in this investigation.

Keywords: Black Gram, Ascorbic Acid, Catalase, Seed Potential, Accelerated Ageing

1. Introduction

Maintenance of seed viability has been a matter of great concern to mankind since the down of the agrarian civilization. High quality seed is the key to successful agriculture. agriculture demands that each and every seed should readily germinate and produce a vigourous seedling ensuring high yield [1]. Storing of seeds is a serious problem in tropical and subtropical countries where high temperature and high relative humidity greatly accelerate seed ageing phenomenon causing consequent deterioration and non-viability of seeds. The problem of retention of seed vigor in many states of India is much more acute because of its semiarid climate where high relative humidity prevailing during the major part of a year is very conducive to the growth of microorganisms, particularly fungi [2, 3]. These two environmental factors strongly impair seed and seedling health and cause to reduce percent seed germinability and seedling performance at a rapid rate [4, 5]. Thus, Indian cultivators are very often compelled to use low vigour seeds in agriculture. To get rid of this problem, strategies are now being undertaken to improve the storage potential of seeds for enhancing their life span [6-8].

Keeping in mind the problem of seed storing, an attempt has been made in this investigation to prolong the storage life of a black gram species. Experiments of this investigation were carried out under accelerated ageing condition to obtain more or less uniform and expeditious results. In fact, accelerated ageing treatment, as imposed by high temperature and high relative humidity (RH), provide a powerful tool for studying the process of seed deterioration over a very short period [9-11].

Thus, the prime objective of this work is to probe the efficacy of the test chemical on enhancement of seed vigour of a black gram species by analysing germination behaviour and metabolic status of the seeds.

2. Materials and Methods

After surface sterilization (0.1% $HgCl_2$ for 90 seconds) the seed sample of black gram (*Vigna mungo* L.) was separately presoaked in aqueous solution of ascorbic acid (100 μg ml^{-1}), or distilled water for 3 hours (h) and then dried back to the original dry weight of the seeds. This was repeated twice allowing maximum penetration of the chemicals present in the aqueous solution. The pretreated seed lot (200 g for each treatment) was taken in separate cloth bag and stored in a desiccator in which

100% relative humidity (RH) was preimposed. This experimental set up was kept at 32±2°C for 30 days allowing the seeds to experience forced ageing treatment.

To analyse the percentage germination, four groups of 100 seeds i.e. 400 seeds of each treatment were transferred to separate Petri dishes containing filter paper moistened with distilled water. Germination data were recorded after 96 h of seed soaking following the International Rules for Seed Testing [12]. The time for 50% germination of seeds (T_{50}) was determined following the method described by Coolbear *et al*. [13].

Protein, insoluble carbohydrate contents as well as activities of catalase and amylase enzymes were analysed from seed kernels. Quantification of insoluble carbohydrates was done following the method of McCready *et al.* [14]. Protein levels was estimated as per the methods of Lowry *et al.* [15]. Extraction and estimation of the enzyme catalase was made following the method of Snell and Snell [16]. Amylase activity was estimated as per the methods of Khan and Faust [17]. For assaying these enzymes, the blank was taken as zero time control and the activity was expressed as (ΔOD x T_V) / (txv), where ΔOD is the difference of OD of the blank and sample. T_V is the total volume of filtrate, t is the time (min) of incubation with the substrate and v is the volume of filtrate taken for incubation [18].

Data were statistically analysed at the treatment and replication levels and least significant difference (LSD) values were calculated at 95% confidence limits [19].

3. Results and Discussion

Results of the experiment clearly revealed that pretreatment of the seed species with aqueous solution of ascorbic acid significantly alleviated the accelerated ageing-induced loss of germination and reduced T_{50} hours (Table 1), slowed down the rapid leaching of insoluble carbohydrates (Table 2), alleviated the loss of protein (Table 2) as well as catalase and amylase (Table 3) enzymes. The chemical-induced substantial amelioration of all these deleterious effects is indicative of seed potentiation under adverse storage environment.

The proposal that a decrease in membrane lesions might play a significant role in deterioration of seeds has been supported by the work on solute leakage accompanying a loss in germinability and viability [20-22]. The ability of seeds to recognize its membrane rapidly as the desiccated tissue rehydrates is a crucial factor for successful germination and this is clearly documented in the literature [23]. Much evidence has been put forward to suggest that membrane status within the germinating embryo is an important factor in deterioration [24]. The results therefore point out that although deterioration is a common phenomenon in treated and control samples of the black gram seed species, the catabolic processes within the treated seed samples remained somewhat subdued, thereby rendering them tolerant against unfavourable storage environment. Available reports show that during seed ageing a loss of some vital cellular components including protein, carbohydrates occurred [25]. Catalase is regarded as a scavenger enzyme and higher activity of this enzyme is indicative of higher plant vigour [26]. In this investigation, the chemical-induced arrestation of rapid loss of the enzyme activity is indicative of strengthening the defence mechanism by the chemical under adverse storage condition.

4. Conclusion

It can be concluded from the results of this investigation that aqueous solution of ascorbic acid is effective in enhancing storage potential of black gram seeds. Thus, invigouration property of the present seed pretreating agent seems to be apparent from these experimental results.

Table 1. *Effect of seed pretreatment with aqueous solution of ascorbic acid (100 µg ml^{-1}) on percentage seed germination and T_{50} hours (time required for 50% germination) values of black gram seeds.*

Seed sample	Treatments	Percentage seed germination			T_{50} of germination		
		Days after accelerated ageing					
		0	15	30	0	15	30
Black gram	Control	100	78	38	12	36	NA
	Ascorbic acid	100	80	52	12	24	84
	LSD (P = 0.05)	NC	5.58	4.38	NC	2.50	6.05

Seeds were presoaked with the aqueous solution of the chemical or distilled water for 6h and then dried back to original seed weight. This was repeated twice. Pretreated seed samples were kept under 100% RH and data were recorded after zero (0), 15 and 30 days of accelerated ageing.
NC: Not calculated; NA: Non attainment of 50% germination.

Table 2. *Effect of seed pretreatment with aqueous solution of ascorbic acid (100 µg ml^{-1}) on protein (mg/g fr. wt.) and insoluble carbohydrates (mg/g fr. wt.) levels of black gram seeds.*

Seed sample	Treatments	Protein			Insoluble carbohydrates		
		Days after accelerated ageing					
		0	15	30	0	15	30
Black gram	Control	61.31	40.33	19.87	23.10	18.50	10.19
	Ascorbic acid	61.09	48.12	28.99	23.16	20.19	17.07
	LSD (P = 0.05)	NS	3.67	1.49	NS	1.07	0.06

Treatments and recording of data as in Table 1.
NC: Not calculated; NS: Not significant.

Table 3. *Effect of seed pretreatment with aqueous solution of ascorbic acid (100 µg ml^{-1}) on activities of enzyme catalase (ΔODxTv/txv) and amylase (ΔODxTv/txv) of black gram seeds.*

Seed sample	Treatments	Catalase			Amylase		
		Days after accelerated ageing					
		0	15	30	0	15	30
Black gram	Control	40.40	26.20	16.90	37.10	50.00	67.80
	Ascorbic acid	40.00	30.90	25.00	37.00	41.20	53.40
	LSD (P = 0.05)	NS	2.20	1.15	NS	3.05	2.70

Treatments and recording of data as in Table 1.
NS: Not significant.

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