

Full Length Research Paper

Seed potentiation of a horse-gram cultivar by herbal manipulation

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An investigation was carried out on seed potentiation of an ethnomedicinal pulse crop cultivar horse-gram by using some selected medicinal plant extracts. Pretreatment of horse-gram seeds with aqueous solution of leaf extracts of *Aegle marmelos* and *Vitex negundo* slowed down the rapid loss of germination. The plant extracts also significantly arrested profuse leakage of amino acids from seeds. Concomitantly, the reduction of protein, insoluble carbohydrate, DNA and RNA levels as well as activity of catalase enzyme of seed kernels during forced ageing period was ameliorated to a significant extent in the plant extract-pretreated seeds. Conversely, ageing-induced stimulation of the activity of amylase enzyme was alleviated by the seed pretreating agents. The promising effects of the experimental plant extracts on storage potentiation of the seeds are apparent in this investigation.

Key words: Seed potentiation, Horse-gram, *Aegle marmelos*, *Vitex negundo*.

INTRODUCTION

Plants have been one of the important sources of medicines even since the dawn of human civilization. In India, the medicinal plants occupy a prominent place in the country's flora. The account of medicinal plants of India was known from the time of Charak (1000 B.C.) and Sushruta (800 B.C.) where they had mentioned nearly 700 species as therapeutic agents and out of which nearly 500 are being mentioned in India Flora. Further it is reported that over 800 plant species are having medicinal value out of 17,000 flowering plants and owing to this India is considered as the Botanical Garden of the World and Treasure House of the Biodiversity. According to WHO (World Health Organization), herbal medicines serve the health care needs of about 80% of the world population. Millions of rural people all over the world use medicinal plants as self-help cure against a number of common diseases. It is also true that the goal of health for all cannot be achieved without green medicines.

Indian farmers are facing various problems in cultivation

of medicinal plants because of lack of agro-technology, inadequate research and post harvest seed storage technique etc. Horse-gram (*Dolichos biflorus* L.) is one of the highly nutritious vegetable pulse crops and has some ethno medicinal values. It is commonly known as Kurti-kalai (Bengali), Horse gram (English) and Kulattha (Sanskrit). The crop grows luxuriantly both in cultivated and widely distributed in India, ascending up to 1000 m in Sikkim; cultivated mainly in Andhra Pradesh, Tamil Nadu, and Karnataka. Traditionally, the aqueous extracts of seed are used to cure urinary troubles, acid peptic disorder (gastritis), constipation, sun-burn, kidney stone, female diseases (leucorrhoea, menstrual troubles, bleeding during pregnancy, post partum excessive discharges), colic caused by wind, piles, rheumatism, hemorrhagic disease, intestinal worms etc. (Laskar et al., 1988; Mishra, 2006). As per Charak Samhita, the seed are useful for the cure of piles, hiccup, abdominal lump, bronchial asthma, in causing and regulating perspiration. It is also mentioned in the Sushruta Samhita that the seed powder is useful in stopping excessive perspiration. It contains essential amino acids having great nutritional value. In spite of its high ethno medicinal and nutritional value the crop is

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Table 1. Effect of seed pretreatment with leaf extracts of *Aegle* sp. and *Vitex* sp. (25g/500ml each) on percentage seed germination and protein (mg/g fr. wt.) levels of horse-gram seeds. Seeds were presoaked with the plant extracts or distilled water for 6h and then dried back to original seed weight. This was repeated twice. Pretreated seed samples were kept under 99.5% RH and data were recorded after zero (0), 15 and 30 days of accelerated ageing.

Seed sample	Treatments	Percentage seed germination			Protein		
		Days after accelerated ageing					
		0	15	30	0	15	30
Horse-gram	Control	100	79	38	71.10	50.21	21.96
	<i>Aegle</i> sp.	100	85	57	71.70	61.80	41.03
	<i>Vitex</i> sp.	100	82	53	71.17	57.82	33.11
	LSD (P = 0.05)	NC	5.60	4.58	NS	4.20	2.17

NC: Not calculated; NS: Not significant.

Table 2. Effect of seed pretreatment with leaf extracts of *Aegle* sp. and *Vitex* sp. (25g/500ml each) on leachate amino acids (mg/g/10ml) and insoluble carbohydrates (mg/g fr. wt.) levels of horse-gram seeds. Treatments and recording of data as in Table 1.

Seed sample	Treatments	Leachate amino acids			Insoluble carbohydrates		
		Days after accelerated ageing					
		0	15	30	0	15	30
Horse-gram	Control	33.0	52.6	67.9	24.10	18.50	10.19
	<i>Aegle</i> sp.	32.2	39.2	42.0	24.17	22.82	18.02
	<i>Vitex</i> sp.	32.7	38.9	48.9	24.16	21.19	17.07
	LSD (P = 0.05)	NS	3.05	4.15	NS	1.01	0.08

NS : Not significant.

not popularized in West Bengal state of India mainly because of its seed viability problem (Mishra, 2006). 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 the year is very conducive to the growth of microorganisms, particularly fungi (Christensen and Kaufmann, 1965; Aziz and Shair, 1997). These two environmental factors strongly impair seed and seedling health and cause to reduce percent seed germinability and seedling performance at a rapid rate (Copeland and McDonald, 1995; Pati and bhattacharjee, 2011). 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 (Chhetri et al., 1993; Basu, 1994) Keeping in mind the problem of seed storing in our country, an investigation

was carried out on enhancement of seed potential of a medicinal pulse crop cultivar horse-gram (*Dolichos biflorus* L. cv BR-5) by using leaf extracts of bel (*Aegle marmelos*) and nishinda (*Vitex negundo*).

MATERIALS AND METHODS

After surface sterilization (0.1% HgCl₂ for 90 seconds) the seed samples of Horse-gram (*Dolichos biflorus* L. cv BR-5) were presoaked in aqueous solutions of leaf extracts of bel (*Aegle marmelos*) and nishinda (*Vitex negundo*) 25g in 500 ml distilled water of each for 6 hours 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 lots (200 g for each treatment) were taken in separate cloth bags and thus stored in a desiccator in which 99.5% relative humidity (RH) was preimposed by keeping 250 ml 1.57% H₂SO₄ within it. This experimental set up was kept at 32±2⁰C for 30 days allowing the seeds to experience forced ageing treatment and H₂SO₄ was changed at 15 day

Table 3. Effect of seed pretreatment with leaf extracts of *Aegle* sp. and *Vitex* sp. (25g/500ml each) on DNA ($\mu\text{g/g}$ fr. wt.) and RNA ($\mu\text{g/g}$ fr.wt.) levels of horse-gram seeds. Treatments and recording of data as in Table 1.

Seed sample	Treatments	DNA			RNA		
		Days after accelerated ageing					
		0	15	30	0	15	30
Horse-gram	Control	48.6	39.2	30.9	127.1	78.2	39.0
	<i>Aegle</i> sp.	48.8	49.1	40.0	127.3	102.1	51.9
	<i>Vitex</i> sp.	48.7	41.4	36.2	127.6	96.7	48.2
	LSD (P = 0.05)	NS	3.27	2.96	NS	7.55	2.99

NS : Not significant.

Table 4. Effect of seed pretreatment with leaf extracts of *Aegle* sp. and *Vitex* sp. (25g/500ml each) on activities of enzyme catalase ($\Delta\text{ODxTv}/\text{txv}$) and amylase ($\Delta\text{ODxTv}/\text{txv}$) of horse-gram seeds. Treatments and recording of data as in Table 1.

Seed sample	Treatments	Catalase			Amylase		
		Days after accelerated ageing					
		0	15	30	0	15	30
Horse-gram	Control	44.0	28.0	18.8	37.9	48.9	69.2
	<i>Aegle</i> sp.	44.1	39.1	32.2	37.0	41.2	50.0
	<i>Vitex</i> sp.	44.0	38.6	28.9	37.7	43.0	52.1
	LSD (P = 0.05)	NS	2.17	1.50	NS	3.28	4.39

NS: Not significant

intervals to restore the desired RH within the desiccator for 30 days. To study the seed potentiation some vital physiological and biochemical parameters viz. percentage seed germination, leachate amino acids, protein, insoluble carbohydrate, DNA and RNA levels as well as activity of catalase and amylase enzymes of seed kernels were analysed. Germination data were recorded following the International Rules for Seed Testing (ISTA, 1976). Quantification of insoluble carbohydrates and amino acid was done following the method of McCready *et al.* (1950) and Moore and Stein (1948) respectively. Protein and nucleic acids (DNA and RNA) levels were estimated as per the methods of Lowry *et al.* (1951) and Cherry (1962) modified by Choudhuri and Chatterjee (1970) respectively. Extraction and estimation of the enzyme catalase was made following the method of Snell and Snell (1971) as modified by Biswas and Choudhuri (1978). Amylase activity was estimated as per the methods of Khan and Faust (1967). For assaying these enzymes, the blank was taken as zero time control and the activity was expressed as $(\Delta\text{ODxT}_v)/\text{txv}$, 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 (Fick and Qualset, 1975).

Data were statistically analysed at the treatment and

replication levels and least significant difference (LSD) values were calculated at 95% confidence limits (Panse and Sukhatme, 1967). 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 (Heydecker, 1972, Pati, 2007, Pati and Bhattacharjee, 2012) and this mimics the natural ageing process

RESULTS AND DISCUSSION

Pretreatment of horse-gram seeds with aqueous solutions of leaf extracts of *Aegle marmelos* and *Vitex negundo* before accelerated ageing treatment slowed down the rapid loss of germination (Table 1). The bioactive chemicals also significantly arrested the profuse leakage of amino acids from seeds (Table 2). concomitantly, the reduction of protein (Table 1), insoluble carbohydrate (Table 2), DNA and RNA (Table 3) levels as well as activity of catalase (Table 4) enzyme of seed kernels during forced ageing period was ameliorated to a significant extent in the plant extracts-pretreated seeds. Conversely, ageing-induced

stimulation of the activity of amylase enzyme (Table 4) was alleviated by the seed pretreating agents. The results therefore point out that although deterioration is a common phenomenon in treated and control samples of the seed species the catabolic processes within the treated seed samples remained somewhat subdued, thereby rendering them tolerant against unfavourable storage environment. In this investigation, the herbal-induced arrestation of rapid loss of the catalase enzyme activity is indicative of strengthening the defence mechanism by the plant extracts under adverse storage condition. The promising effects of the experimental plant extracts on storage potentiation of the seeds are apparent in this investigation.

Achievements of the Work

- a. Accelerated ageing technique efficiently and accurately determined deterioration level of seeds in a short period under storage.
- b. The herbal manipulation technique is effective for maintenance of storage potential of horse gram seeds for longer period.
- c. The test plant extracts can harden seeds thereby causing less susceptible towards deterioration particularly under accelerated ageing condition.
- d. The technique devised is very simple, handy and eco-friendly, cost-effective for common cultivators.

CONCLUSION

Considering the herbal manipulated over all changes on germination behaviour, metabolism and growth it can be concluded that the aqueous solution of the herbal extracts can be used as a potent agents for maintaining the seed vigour of the horse-gram cultivar under stressful storage situation. Thus, the work has immense significance from commercial point of view in addition to some academic interest.

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