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RESEARCH ARTICLE

SEED INVIGORATION TREATMENTS EMPLOYING ECOFRIENDLY WET AND DRY POWDERED BOTANICALS ALONG WITH PHARMACEUTICALS FOR IMPROVED STORABILITY AND FIELD PERFORMANCE OF HIGH-MEDIUM VIGOUR WHEAT (*Triticum aestivum* L.)

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ABSTRACT

Hydration-dehydration treatments (soaking-drying and moist sand conditioning drying) and dry-dressing treatments especially with red chilli powder (*Capsicum frutescens* L.) @ 1g/kg of seeds and ascorbic acid @ 500mg/kg of seeds were effective in slowing down seed deterioration of high-medium vigour (3-month-old seed) wheat (cv. PBW-343) seed under subsequent ageing conditions. The wet treatments viz., soaking-drying and moist sand conditioning drying significantly improved germinability and field performance and productivity (effective tiller/ m^2, grain yield/ m^2 and 1000-seed weight) over untreated control. A few dry treatments, especially, red chilli powder and ascorbic acid also showed better results in improving storability and field performance than the untreated control. But the major effects were due to wet treatments in maintaining germinability as well as field performance of high-medium vigour wheat seeds. Physiological and biochemical studies revealed that soaking drying and moist sand conditioning drying followed by few dry treatments like red chilli powder and ascorbic acid showed reduced leakage of electrolytes and sugars with lower lipid peroxide formation and higher dehydrogenase enzyme activity than the control. On the basis of the above findings, wet treatments viz., soaking drying and moist sand conditioning drying; and dry-dressing treatment with red chilli powder may be suggested for the improvement of vigour, viability and field performance of high-medium vigour wheat seeds.

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INTRODUCTION

In the coastal belts of our country especially in eastern India, availability of good quality seeds as a planting material is difficult, because preservation under ambient conditions is a serious problem due to high humidity and high temperature where the average relative humidity and temperature are about 80% and 30°C, respectively throughout the year. High atmospheric humidity coupled with prevailing high temperature during a major part of the seed storage period greatly hastens physiological seed-ageing and as a result, the viability goes down to half or even less at the time of planting in November – December. The fall in germination percentage of wheat seed during subsequent storage under ambient conditions may be compensated by corresponding seed rate adjustment but how the loss of vigour could be compensated by seed rate adjustment. Hydration-dehydration treatment of stored medium- to low-vigour seeds of agriculturally important orthodox crop plants would greatly minimize their physiological deterioration during subsequent storage under uncontrolled warm humid conditions (Mandal and Basu, 1986). Mandal *et al*. (1999) reported that the dry seed treatments in harvest fresh seed with halogen compound, pharmaceutical formulations and crude plant materials significantly slowed down seed deterioration of a number of agricultural and horticultural crop seeds (Mandal *et al*., 1999, 2000; De *et al*., 2003; Guha *et al*., 2013). Considering the above circumstances, standardization of an easily practicable method of seed invigoration treatments were taken up for the retention of vigour, viability and field performance of high-medium vigour wheat seeds.

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MATERIALS AND METHODS

Wheat seeds (3-month-old) cv. PBW-343 were collected from the Calcutta University Experimental Farm at Baruipur, South-24-parganas, West Bengal. After collection, seeds were dried to a moisture content of about 9.8% (ISTA, 1996) for safe storage in rubber stoppered air-tight glass bottles under ambient conditions in the laboratory till they were used for treatments. The experiments were conducted in two consecutive seasons.

Methods of seed treatment

Dry treatments

Wheat seeds (3-month-old) were dry-dressed with finely powdered pharmaceutical formulations (aspirin @ 50 mg/ kg of seed and ascorbic acid @ 500mg/kg of seed) and crude plant materials (red chilli powder @ 1g/ kg of seed and neem leaf powder @ 2g/ kg of seed) in the rubber stoppered glass bottles at room temperature (29±1^0C). After treatment, glass bottles were shaken once in a day upto 7 days for through mixing of treatment with the seeds and then stored under ambient conditions.

Hydration-dehydration treatment

In soaking-drying treatment, wheat seeds were soaked in double volume of water for 2 hours at room temperature (29±1^0C) with occasional stirring followed by drying back to its original moisture content in the sun or artificial dehumidified drying chamber. In moist sand conditioning and drying (MSC-D) treatment, pre-washed air-dried sand were moistened with distilled water (6%) and then wheat seeds were mixed thoroughly with the pre-moistened sand in the ratio 1 : 3 and kept covered for 24 h at room temperature. After conditioning, seeds were dried back to its original moisture content. All the treated and untreated seeds were further stored in 500ml capacity rubber stoppered glass bottles under ambient conditions until sowing in the field. After 15 days of treatment, treated and untreated seeds were subjected to natural ageing by taking the seeds separately in perforated paper packets (containing same amount of seed with equal number of holes) and then all the packets were kept in a cloth bag under ambient conditions (average RH 82±1.78% and temperature 30±1.6°C) for 90 days. The packets were shaken at regular intervals for uniform ageing. Germination test of treated and untreated seeds (more than 400 seeds for each treatment as specified by ISTA, 1996) were done following the method of Punjabi and Basu (1982) with minor modifications. Data on germination percentage and seedling length were recorded 5 days after germination. During the measurement of root length, a total of all seminal roots per seedling were taken.

Field experiment

Field performance was carried out during *rabi* season, using randomized block design with 3 replications for each treatment. After land preparation, field was divided into 3 blocks and each block containing 7 subplots of $10m^2$. A fertilizer dose of $N:P_2O_5:K_2O$ was applied @ 80:40:40 kg / ha respectively. During final land preparation, 50% nitrogen and whole amount of phosphorous and potassium were added. The rest of the nitrogen was added in two split doses. Seeds were sown @ 100 kg/ ha and sowing was done on the mid of November in two consecutive years 2015-16 and 2016-17 giving a spacing of 40 cm between rows and 25 cm between the plants. A post sowing irrigation was made on the same day. Besides, three more irrigations and other cultural practices were done throughout the cropping period.

Physiological and biochemical studies

To study membrane permeability of treated and untreated seeds, the electrical conductance and sugar of the seed leachate was measured following the method of Anderson *et al*. (1964) and McCready *et al*. (1950) respectively. Thirty seeds from each treatment were soaked in 30 ml of distilled water for 2h at 28 ± 1°C and then seed steeped water was decanted off and electrical conductance of seed leachate was recorded on a Conductivity Bridge (cell constant = 0.756). The amount of sugar was determined by adding 4 ml of ice-cold freshly prepared anthrone reagent (0.2% anthrone in 98% sulphuric acid) to 2 ml of pre-cooled seed leachate in a hard glass test tube and kept in cold for 30 minutes for the development of bluish green colour. The intensity of the colour was measured on a Photoelectric Colorimeter at 580nm. Lipid peroxide formation of treated and untreated seeds was estimated following the method of Bernheim *et al*. (1948) with minor modifications. Five ml of 1% TBA reagent and 2ml of 1N sulphuric acid was added to 100mg powdered seed. The mixture was heated for 15 minutes in a water bath at 100°C. After cooling, 5ml of 2-methoxy ethanol were added and shaken and then the mixture was centrifuged at 10,000rpm for 10 minutes and the intensity of colour was then measured by systronics spectrophotometer at 520nm. The dehydrogenase enzyme activity of treated and untreated seeds were estimated following the method of Kittock and Law (1968). Eight sprouted embryos were dipped in 5 ml of 0.2% tetrazolium chloride solution and incubated for 3h in the dark at 28 ± 1°C. After incubation, the solution was decanted off and the embryos were thoroughly washed with distilled water and surface dried. Then five ml of 2-methoxy ethanol were added and kept for 3h for the extraction of red colour formazan. The absorbance of the solution was recorded on a Photoelectric Colorimeter at 470nm. Data on plant population was recorded after 15 days of sowing. The plant height was measured after completion of vegetative growth of the crop. Data on number of effective tillers per m^2, panicle length, number of seeds per panicle, grain yield per m^2 and 1000-seed weight were taken after harvesting of the crop. Data obtained from laboratory and field experiment were analyzed statistically to evaluate the treatment effects on yield and yield attributes of wheat following the method of analysis of variance (Fisher, 1948).

RESULTS AND DISCUSSION

Germination tests conducted immediately after treatment did not show any significant difference between treated and untreated seeds in improving germinability (Table 1). But, after natural ageing under ambient conditions for 90 days, most of the treated seeds showed significant beneficial effect on germination percentage and seedling growth as measured by root and shoot length over untreated control (Table 1). Among the treatments, wet treatments (soaking-drying and moist sand conditioning drying) alongwith a few dry treatments (red chilli powder, ascorbic acid and neem leaf powder) showed significant improvement in extending storability than the untreated control. Besides, vigour index as calculated by germination percentage multiplied by seedling length was higher in the wet treatment than the untreated control and dry treatments. The crop raised from the high-medium vigour treated and untreated seeds (cv. PBW-343), showed that plant population, number of effective tillers per m^2, plant height, length of panicle, number of seeds per panicle, grain yield per unit area and 1000-seed weight was significantly improved by the wet (soaking-drying and moist sand conditioning drying) as well as dry treated seeds over control (Table 2). Among the treatments, soaking drying and moist sand conditioning drying

Table 1. Effect of seed invigoration treatments for the maintenance of vigour and viability of wheat (cv. PBW -343) seeds immediately after treatment (before ageing) and after natural ageing under ambient conditions for 90 days (average 72 ±3.2 % RH and 29 ± 1.6 ^{o} C)

Treatments	Before ageing					Natural ageing				
	Germination		Mean root length (mm)	Mean shoot length(mm)	Vigour Index	Germination		Mean root length (mm)	Mean shoot length(mm)	Vigour Index
	%	Arc-Sin Value				%	Arc-Sin Value			
Control	89	70.86	342	41	34087	50	44.91	205	24	11450
Aspirin	90	71.32	355	46	36090	50	44.74	19 9	31	11500
Red chilli powder	90	71.80	361	42	36270	55	47.90	248	32	15400
Neem leaf powder	90	70.79	356	42	35820	53	46.64	234	29	13939
Ascorbic acid	90	71.24	307	42	31410	55	47.78	242	31	15015
Soaking-drying (S-D)	91	72.20	371	47	38038	64	53.04	265	34	19136
Moist sand conditioning drying (MSC-D)	90	71.90	364	47	36990	61	51.44	254	33	17507
L.S.D at 0.05 P	-	NS	NS	NS	-	-	2.1	22.3	2.3	-

Abbreviation: - NS - Non-Significant

Treatments were given to 3-month-old seeds of wheat. Seeds of wheat were dry dressed with finely powdered pharmaceuticals (aspirin @ 50mg/kg of seed and ascorbic acid @ 500 mg/ kg of seed), and air dried powdered crude plant materials (Red chilli powder@ 1 g/kg of seed, Neem leaf powder @ 2g /kg of seed) in the rubber stoppered glass bottles at room temperature (29 ^{o}± 1°C). In case of wet treatment, seeds were soaked in double volume of water for 2h and moist sand conditioning for 24h followed by drying to its original weight.

Data was recorded after 5 days of germination.

Vigour Index :- Germination percentage (%) × Seedling Length

Table 2. Effect of seed invigoration treatments on field performance and productivity of wheat (cv. PBW -343)

Treatments	Plant population/ m^2	No. of effective tillers / m^2	Plant Height (cm)	Panicle length (mm)	No. of seeds / panicle	Grain yield/m^2 (g)	1000 seed weight (g)
Control	128	385	80	81	22	207.63	26.03
Aspirin	119	358	82	81	23	212.77	27.20
Red chilli powder	133	403	85	88	28	233.33	29.06
Neem leaf powder	127	382	84	84	26	216.80	29.05
Ascorbic acid	131	422	86	85	25	232.92	25.51
Soaking-drying (S-D)	149	455	91	98	29	256.25	32.55
Moist sand conditioning drying (MSC-D)	147	428	91	94	28	255.02	35.10
L.S.D at 0.05 P	8	6	3	3.5	1.2	5.4	1.8

Other details are same as Table 1.

Table 3. Effect of seed invigoration treatments on germination percentage (arc-sin value), membrane permeability, enzyme activity and lipid peroxidation of wheat immediately after treatment (before ageing) and after natural ageing under ambient conditions for 90 days (average 72 ± 3.2 % RH and 29 ± 1.6 ^{o} C)

Treatments	Before ageing					Natural ageing				
	Germination (%)	Electrical conductivity (μscm^2)	Leaching of sugar (μg/ml)	Dehydrogenase activity (O.D value)	Lipid peroxidation (O.D value)	Germination (%)	Electrical conductivity (μscm^2)	Leaching of sugar (μg/ml)	Dehydrogenase activity (O.D value)	Lipid peroxidation (O.D value)
Control	80 (63.4)*	30.12	16.55	0.538	0.06	50 (45.0)*	108.23	40.35	0.30	0.29
Aspirin	72 (58.1)	26.07	12.57	0.540	0.05	50 (45.0)	95.53	46.05	0.31	0.27
Red chilli powder	82 (64.9)	25.13	11.52	0.542	0.05	60 (50.8)	62.37	30.43	0.34	0.24
Neem leaf powder	78 (62.0)	30.16	17.48	0.548	0.08	61 (51.4)	75.42	35.99	0.32	0.25
Ascorbic acid	78 (62.0)	33.16	12.54	0.546	0.04	62 (51.9)	66.06	30.52	0.33	0.25
Soaking-drying (S-D)	85 (67.2)	25.10	10.50	0.549	0.03	(58.1) 72	54.23	25.35	0.37	0.15
Moist sand conditioning drying (MSC-D)	82 (64.9)	25.07	12.26	0.546	0.03	70 (56.8)	58.92	28.43	0.35	0.17
L.S.D at 0.05 P	NS	NS	NS	NS	NS	4.5	0.9	1.01	0.01	0.03

* Data in parentheses indicate the arc-sin transferred values and figures without parentheses indicate the original values.
Other details are same as Table 1.

followed by a few dry treatment viz., red chilli powder and ascorbic acid gave better results in improving field performance and productivity (Table 2). The major beneficial effects were due to wet treatments in increasing yield and other yield attributes of high-medium vigour wheat seeds. Biochemical tests of treated and untreated seeds carried out immediately after treatments did not show any noticeable difference on membrane integrity and enzyme activity (Table 3). But after natural ageing for 90 days, the membrane permeability of treated seeds showed significantly lower electrical conductance of seed leachate and leakage of sugar with lower lipid peroxide formation and higher dehydrogenase enzyme activity than the untreated control (Table 3). Among the treatments, soaking drying and moist sand conditioning drying followed by dry treatments viz., red chilli powder, ascorbic acid and neem leaf powder were much effective in reducing the leakage of electrolytes and sugars along with lower lipid peroxide formation and higher dehydrogenase enzyme activity than the control (Table 3). The wet treatments viz., soaking-drying and MSC-D has shown better results in maintaining membrane integrity, lower lipid peroxide formation and higher dehydrogenase enzyme activity than the untreated control and dry treatments (Table 3).

In the present study, the efficacy of treatment was noted only after sufficient physiological ageing of seeds upon storage. A number of inexpensive and easily practicable methods of seed treatments have been developed in the present laboratory by Basu, Mandal and other co-workers (Basu, 1994; Mandal *et al.*, 2000; De *et al.*, 2003; and Guha *et al.*, 2012) of which hydration-dehydration treatment of medium- and low-vigour leguminous and non-leguminous crop seeds proved most effective in terms of reproducible as well as yield attributes in a wide variety of crops. Two possibilities viz., the involvement of the cellular repair system in correcting age-induced biochemical lesions during seed hydration (Villiers and Edgcumbe, 1975; Burgass and Powell, 1984) and control of free radical and lipid peroxidation reactions by hydration-dehydration treatment (Basu, 1994; Berjak, 1978; Rudrapal and Basu, 1979; Buchvarov and Gantcheff, 1984). Wilson and McDonald (1986) suggested that seed deterioration would take place during ageing via lipid peroxidation. Dry physiological treatments with pharmaceutical formulations and crude plant materials in harvest-fresh seeds are effective in slowing down seed deterioration of soybean, safflower, blackgram and okra (De *et al.*, 2004; Kapri *et al.*, 2005; Mandal *et al.*, 2000; and Guha *et al.*, 2012) in order to circumvent the problem of drying back (for re-storage) encountered in 'wet' treatments hold considerable promise because of their eco-friendly and easy-to-do approaches. Capsaicin, an active ingredient of red chilli powder is acknowledged as an inhibitor of lipid peroxidation (Brand *et al*., 1990; Dey and Ghosh, 1993). Free radical and lipid peroxidation reactions in the stored seed (Buchvarov and Gantcheff, 1984; Dadlani and Agrawal, 1983; Wilson and McDonald, 1986; Basu, 1994; McDonald, 1999) may be one of the reason of seed deteriorartion. The antioxidants present in crude plant materials and pharmaceuticals viz., neem leaf powder (active ingredient, azadirachtin) and vitamin C contained in ascorbic acid (used as an antioxidant) respectively proved better results in maintaining viability of seeds. The mechanism of entry of dry active ingredient into the dry-stored seeds requires critical elucidation. Cracks and crevices in the seed may serve as entry points of exogenously applied substances. On the basis of the present results, wet treatments viz., soaking-drying and moist sand conditioning drying are advocated for the maintenance of germinability and field performance of high-medium vigour wheat seeds. If the seed drying conditions are not good then dry treatments with red chilli powder @ 1 g/kg of seed and ascorbic acid @ 500 mg/kg of seed may be practiced for improved storability and field performance of high-medium vigour wheat seed.

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