Seed Research, Vol. 44(1): 70-75, 2016

Effect of Seed Colouring with Natural and Artificial Dyes on Storability of Black Gram Seeds

S DAS, S MOHANTY* AND S K SWAIN

Department of Seed Science and Technology, OUAT, Bhubaneswar 751 003
*simantamohanty@yahoo.com

ABSTRACT: An investigation was undertaken in the Seed Physiology Laboratory, Department of Seed Science and Technology, College of Agriculture, Bhubaneswar, to study the effect of seed colouring with few artificial and natural dyes on storability of black gram seeds. Freshly harvested black gram seeds were treated with fungicide (Thiram @ 2g per kg seed), followed by colouring with five artificial dyes @ 0.75% concentration, viz. Aniline blue, Congo red, Methyl violet, Bromocresol purple and Coomasie brilliant blue, and four natural dyes, viz. Beet (root tuber extract), Turmeric (dried rhizome powder), Mehndi (leaf extract), Marigold (extract from petals). One control treatment was also taken, in which only fungicide treatment was given. After colouring, the seeds were dried to moisture content below MSCS (9.0%) and stored in cloth bags under ambient conditions, for a period of 8 months, i.e. from October 2014 to May 2015. Most of the dye treatments, both artificial and natural, had some deleterious effect on maintenance of viability in seeds, as compared to control, though in some cases, the differences were statistically non-significant. None of the dyes had any beneficial effect on germination or vigour parameters. Among the artificial dyes, least deleterious effect was observed in case of seeds treated with Bromocresol purple and Congo red, in terms of various physiological parameters like germinability, percent abnormal seedlings, seed vigour indices and field emergence. Among natural dyes, Mehndi and Marigold treated seeds gave better results than Beet and Turmeric treated seeds, in terms of the physiological parameters. However, seeds coloured with Turmeric showed significantly lower insect infestation throughout the period of storage, as compared to the other treatments and control, clearly indicating its role in control of insect infestation during storage. Maximum deleterious effect of seed physiological parameters was recorded in case of Aniline blue and Methyl violet.

Key words: Seed colouring, Seed quality, Seed storability, Black gram

INTRODUCTION

Among various seed enhancement techniques, seed colouring, or the practice of providing an exogenous colour coating to seeds, started as a necessary practice in the developed countries to avoid the possibility of accidental use of treated seeds as food or feed. Colouring of seed has several merits like, improving seed marketability, improving the appearance of a lot in case of seed discolouration, enabling brand identification, acting as a visual means of ensuring uniformity of seed treatment, enabling farmers for easy identification of varieties based on colour, acting as insect and bird repellent, and checking adulteration by giving different colours to different batches of seeds. In India, seed colouring is only of relatively recent interest and is still considered by many as of lesser importance than the other enhancement techniques or even as an unnecessary and extravagant exercise.

Colouring of seeds is done by use of artificial dyes or natural colouring pigments. Some of the natural pigments and artificial dyes available in the market may have deleterious effect on seed storability and its subsequent performance. A few workers have studied the effect of dyes on the seed quality. The effect of seed colouring on the quality of soybean and tomato seeds was studied by Tonapi *et al.* [1] encompassing 25 dyes at

Received : February 2016 Revised : April 2016 Accepted : April 2016

0.75% concentration and concluded that the dyes Rhodamine-B, Fast green and Malachite green were the best dyes for soybean seed. For tomato seeds, Rhodamine-B and Fast green were found to have least deleterious effect on the seed quality. From a similar experiment, Tonapi *et al.* [2] reported that the dyes Rhodamine-B, Fast green and Fuchsine, in order of preference, were found to be the best among all dyes in having minimum deleterious effect on both paddy and maize seeds. Similarly, the dyes Rhodamine-B and Erichro black-T in castor, Rhodamine-B and Cotton blue in sunflower and Rhodamine-B, Fuchsine and Neutral Red in safflower had the least deleterious effect on seed quality during storage and its subsequent performance [3]. Harinath Babu *et al.* [4] reported that the dyes, Rhodamine-B, Fuchsine and Titan yellow for red gram, Rhodamine-B, Fuchsine and Phenol red for black gram and Rhodamine-B, Crystal violet and Titan yellow for Bengal gram were found to be the best dyes for seed colouring at 0.75% concentration. Colouring of seeds with green herbal textile dye + insecticide treatment has been reported to control rice weevil infestation in hybrid sorghum [5]. Though the above-mentioned studies have thrown light on the effectiveness of seed colouring on the storability and performance of seeds, further research needs to be undertaken to substantiate the above results, encompassing more number of crops and by using more number of natural or artificial dyes. Considering the above discussions, the present study was undertaken to study the effect of seed colouring on the physiological properties, in a few crop seeds and identify various dye (s) suitable for colouring of different crop seeds *vis à vis* seed storability.

MATERIALS AND METHODS

Freshly harvested black gram seeds were treated with fungicide (Thiram @ 2g per kg seed), followed by colouring with five artificial dyes @ 0.75% concentration, viz. Aniline blue (T_1), Congo red(T_2), Methyl violet (T_3), Bromocresol purple (T_4) and Coomasie brilliant blue (T_5), and four natural dyes, viz. Beet (root tuber extract) (T_6), Turmeric (dried rhizome powder) (T_7), Mehndi (leaf extract) (T_8), Marigold (extract from petals) (T_9). One Control (T_{10}) was also taken, in which only fungicide treatment was given. After colouring, the seeds were dried to moisture contents below Indian Minimum Seed Certification Standards for black gram (9%) and stored in cloth bags under ambient conditions for a period of 8 months, i.e., from October 2014 to May 2015. The experiment was laid out in Completely Randomised Design with three replications. Observations on seed moisture content (%), germination (%), Seed Vigour Index-I [6], speed of germination, germination after accelerated ageing (%), field emergence (%), infected seeds (%) and insect infestation (%) were recorded at monthly intervals. Data obtained from the experiment were analysed using suitable statistical techniques.

RESULTS AND DISCUSSION

Seed colouring with artificial and natural dyes had little effect on the seed moisture content during storage. The variation among the treatments was found to be statistically non-significant (Table 1). However, the mean seed moisture content (over 8 months) was slightly higher in T_7 (Turmeric), the difference with other treatments being non-significant.

Seed germination among all the treatments decreased gradually with the increase in storage period. The germination potential of T_{10} (Control), i.e. seed treated with fungicide only and without any colouring, was higher over 8 months of storage (Table 1). Among the artificial dyes, T_4 (Bromocresol purple), T_2 (Congo red) and T_5 (Coomasie brilliant blue) proved to have least deleterious effect on germination potential of the seeds. Maximum deleterious effect was observed in case of T_3 (Methyl violet) and T_1 (Aniline blue).

The highest mean shoot and root lengths were observed in case of T_{10} (control), thus indicating that all the treatments had slight deleterious effect on maintenance of seed quality during storage. Lowest shoot length was recorded in case of T_8 (Mehndi), while lowest root length was observed in case of T_1 (Aniline blue) and T_6 (Beet). In contrast to all treatments, highest seedling dry weight was recorded in case of T_{10} (Control) and

T_8 (Mehndi), whereas lowest value was recorded in case of T_5 (Coomasie brilliant blue) (Table 1 and 2).

In comparison to all the dye treatments, T_{10} (Control) recorded the highest Seed Vigour Index-I and SVI-II values, followed by T_5 (Coomasie brilliant blue) (Table 2). Low Seed Vigour Index values were recorded in case of T_1 (Aniline blue) and T_3 (Methyl violet). The two treatments also recorded the highest percent decrease in Seed Vigour Index values over 8 months of storage, thus suggesting some deleterious effect of the two treatments of seed viability maintenance during storage.

The per cent infected seeds were found to be higher in T_{10} (Control) as well as all the natural dyes, as compared to the artificial dyes, clearly indicating that the artificial dyes supplemented the fungicide treatment in controlling the pathogens to a greater extent (Table 3). Lowest percentage of infected seeds was recorded in case of T_2

Table 1 : Moisture content and germination parameters of black gram seed over 8 months of storage under ambient condition as influenced by seed colouring

Treatment	Moisture content (%)		Germination (%)			Abnormal seedlings (%)			Seedling shoot length (cm)	
	Start of storage (0 month)	After 8 months storage	Start of storage	After 8 months	% change during 8 months	Start of storage (0 month)	After 8 months	% change during 8 months	Start of storage	After 8 months
T_1 : Aniline blue	8.11	9.06	82.50(9.08)*	70.50(8.40)*	-14.5	5.50(2.45)*	8.50(3.00)*	54.5	18.45	16.18
T_2 : Congo red	8.71	8.92	81.75(9.04)*	77.75(8.82)*	-4.9	5.50(2.45)*	8.75(3.04)*	59.1	17.20	15.08
T_3 : Methyl violet	8.31	9.18	82.00(9.06)*	70.25(8.38)*	-14.3	5.00(2.35)*	8.25(2.96)*	65.0	19.98	17.34
T_4 : Bromocresol purple	8.74	8.87	82.25(9.07)*	78.00(8.83)*	-5.2	5.50(2.45)*	9.00(3.08)*	63.6	18.96	16.29
T_5 : Coomasie brilliant blue	8.34	8.65	82.00(9.06)*	75.75(8.70)*	-7.6	5.25(2.40)*	8.75(3.04)*	66.7	18.54	16.09
T_6 : Beet (*Beta vulgaris*)	8.53	8.96	82.50(9.08)*	72.00(8.49)*	-12.7	5.00(2.35)*	8.25(2.96)*	65.0	19.04	16.35
T_7 : Turmeric (*Curcuma longa*)	8.92	9.32	82.25(9.07)*	72.75(8.53)*	-11.6	5.00(2.35)*	8.50(3.00)*	70.0	18.89	16.23
T_8 : Mehndi (*Lawsonia inermis*)	8.38	9.18	82.75(9.10)*	73.50(8.57)*	-11.2	5.25(2.40)*	8.25(2.96)*	57.1	18.22	15.82
T_9 : Marigold (*Tagetes erecta*)	8.41	9.33	82.25(9.07)*	76.75(8.76)*	-6.7	5.50(2.45)*	8.50(3.00)*	54.5	17.26	14.98
T_{10} : CONTROL	8.29	9.02	82.75(9.10)*	79.00(8.89)*	-4.5	5.50(2.45)*	8.25(2.96)*	50.0	18.12	15.72
S.E.m (±)	0.418	0.347	0.068	0.141		0.042	0.055		0.537	0.644
$C.D._{0.05}$	NS	NS	NS	0.417		NS	NS		1.583	1.899
CV	2.86	2.83	2.88	2.21		1.82	2.63		1.54	2.74

* Figures in the parenthesis are square root transformed values [$y = \sqrt{x}$]

Table 2 : Germination and vigour parameters of black gram seed over 8 months of storage under ambient condition as influenced by seed colouring

Treatment	Seedling root length (cm)		Seedling dry weight (g)		Seed Vigour Index-I			Seed Vigour Index-II		
	Start of storage	After 8 months	Start of storage	After 8 months	Start of storage	After 8 months	% change during 8 months	Start of storage	After 8 months	% change during 8 months
T_1 : Aniline blue	5.88	5.16	0.912	0.799	2007.2	1504.1	-25.1	75.20	56.35	-25.1
T_2 : Congo red	6.45	5.66	0.942	0.818	1933.4	1612.4	-16.6	77.04	63.60	-17.5
T_3 : Methyl violet	9.30	8.15	0.911	0.791	2400.6	1791.0	-25.4	74.73	55.57	-25.6
T_4 : Bromocresol purple	10.07	8.65	0.880	0.764	2387.7	1945.7	-18.5	72.39	59.59	-17.7
T_5 : Coomasie brilliant blue	10.49	9.11	0.901	0.782	2380.1	1908.5	-19.8	73.91	59.26	-19.8
T_6 : Beet (*Beta vulgaris*)	7.59	6.52	0.904	0.784	2196.6	1647.1	-25.0	74.55	56.47	-24.3
T_7 : Turmeric (*Curcuma longa*)	10.50	9.02	0.924	0.794	2416.5	1836.6	-24.0	76.00	57.76	-24.0
T_8 : Mehndi (*Lawsonia inermis*)	9.24	8.02	0.930	0.816	2272.3	1752.0	-22.9	76.97	59.95	-22.1
T_9 : Marigold (*Tagetes erecta*)	7.64	6.63	0.955	0.837	2047.2	1658.3	-18.1	78.54	64.27	-18.2
T_{10} : CONTROL	10.38	9.01	0.913	0.792	2357.5	1953.7	-17.1	75.52	62.58	-17.1
S.E.m (±)	0.691	0.945	0.0145	0.0217	108.88	91.05		1.32	1.40	
$C.D._{0.05}$	2.038	2.787	0.0429	0.0639	321.19	268.59		3.89	4.14	
CV	2.98	3.28	2.86	2.76	3.16	2.64		2.66	3.11	

(Congo red), followed by T_5 (Coomasie brilliant blue).

Among all the treatments, least insect infestation was recorded in case of T_7 (Turmeric), followed by T_9 (Marigold) (Table 3). The treatment T_7 (Turmeric) was found to produce significantly lower percentage of insect infestation throughout the storage period. Among the artificial dyes, T_4 (Bromocresol purple) gave better result with regard to controlling the storage insects.

In comparison to the dye treatments, highest germination percentage after accelerated ageing was recorded in case of T_{10} (Control), clearly indicating some deleterious effect of the treatments on seed quality during storage (Table 3). Among the treatments, T_4 (Bromocresol purple) and T_9 (Marigold) gave good germination after accelerated ageing, while T_1 (Aniline blue) and T_3 (Methyl violet) recorded the least germination value.

Highest field emergence was recorded in case of T_{10} (Control), while all the dye treatments showed a slight deleterious

effect on the seeds (Table 3). Among the treatments, best results were given by T_9 (Marigold), followed by T_4 (Bromocresol purple). Lowest field emergence values were observed in case of T_1 (Aniline blue) and T_3 (Methyl violet), clearly indicating maximum deleterious effect on the seeds, in conformation with the observations on germination percentage after accelerated ageing.

Hence, to summarise the experiment, it can be mentioned that all the dye treatments, both artificial and natural, had slight deleterious effect on storability of black gram seeds, as compared to Control, though in some cases the differences were statistically non-significant. None of the dyes had any beneficial effect on germination or vigour parameters. However, among the artificial dyes, least deleterious effect was observed in case of seeds treated with Bromocresol

Table 3 : Storability and performance of black gram seeds over 8 months of storage under ambient condition as influenced by seed colouring

Treatment	Infected seeds (%)		Infected seeds (%)		Germination (%) after AA			Field emergence (%)		
	Start of storage	After 8 months	Start of storage	After 8 months	Start of storage	After 8 months	% change during 8 months	Start of storage	After 8 months	% change during 8 months
T_1 : Aniline blue	0.00(0.71)**	3.00(1.87)**	0.00(0.71)**	0.75(1.12)**	62.50	45.50	-27.2	72.25(8.50)*	59.50	-17.7
T_2 : Congo red	0.00(0.71)**	2.25(1.66)**	0.00(0.71)**	1.00(1.22)**	61.25	50.25	-17.9	71.50(8.46)*	65.75	-8.0
T_3 : Methyl violet	0.25(0.87)**	2.50(1.73)**	0.00(0.71)**	1.25(1.32)**	62.25	45.75	-26.5	71.75(8.47)*	59.50	-17.1
T_4 : Bromocresol purple	0.00(0.71)**	2.75(1.80)**	0.00(0.71)**	1.00(1.22)**	62.75	50.50	-19.5	72.00(8.49)*	66.00	-8.3
T_5 : Coomasie brilliant blue	0.50(1.00)**	3.00(1.87)**	0.00(0.71)**	1.25(1.32)**	62.25	48.75	-21.7	71.75(8.47)*	64.00	-10.8
T_6 : Beet (*Beta vulgaris*)	0.00(0.71)**	3.25(1.94)**	0.00(0.71)**	1.50(1.41)**	62.25	46.75	-24.9	72.25(8.50)*	61.00	-15.6
T_7 : Turmeric (*Curcuma longa*)	0.00(0.71)**	2.75(1.80)**	0.00(0.71)**	0.50(1.00)**	62.25	47.25	-24.1	72.00(8.49)*	61.50	-14.6
T_8 : Mehndi (*Lawsonia inermis*)	0.00(0.71)**	3.50(2.00)**	0.00(0.71)**	1.25(1.32)**	62.50	47.50	-24.0	72.50(8.51)*	62.00	-14.5
T_9 : Marigold (*Tagetes erecta*)	0.00(0.71)**	2.75(1.80)**	0.00(0.71)**	1.50(1.41)**	62.00	49.25	-20.6	72.00(8.49)*	65.00	-9.7
T_{10} : CONTROL	0.00(0.71)**	2.50(1.73)**	0.00(0.71)**	1.25(1.32)**	62.75	51.25	-18.3	72.50(8.51)*	66.75	-7.9
S.E.m (±)	0.193	0.212	0.000	0.091	0.713	1.233		0.053	1.101	
$C.D._{0.05}$	NS	NS	NS	0.268	NS	3.636		NS	3.248	
CV	1.86	2.45	0.00	2.43	3.28	2.93		3.12	1.98	

*Figures in the parenthesis are square root transformed values [$y = \sqrt{x}$]; **Figures in the parenthesis are square root transformed values [$y = \sqrt{(x + 0.5)}$]

purple and Congo red, in terms of various physiological parameters like germinability, per cent abnormal seedlings, seed vigour indices and field emergence. Among natural dyes, Mehndi and Marigold treated seeds gave better results than the Beet and Turmeric treated seeds, in terms of the physiological parameters. However, seeds coloured with Turmeric showed significantly lower insect infestation throughout the period of storage, as compared to the other treatments and Control, clearly indicating its role in control of insect infestation during storage. Maximum deleterious effect of seed physiological parameters was recorded in case of Aniline blue and Methyl violet.

Considering the above findings from the investigation, it can be concluded that among artificial dyes, Bromocresol purple and Congo red can safely be recommended for colouring of black gram seeds. In case of natural dyes, Mehndi leaf extract and Marigold petal extract proved to be good options for colouring of black gram seeds. However, further investigations encompassing several other dyes may be taken up to have better screening of the artificial and natural dyes, as well as to fix seed colouring standards. Similarly, calculating the cost of dye treatment and benefits arising out of it may need further study.

REFERENCES

1. TONAPI VA, P HARINATH BABU, NA ANSARI, S VARANAVASIAPPAN, CH RAVINDER REDDY, SS NAVI AND N SEETHARAMA (2006a). Studies on seed colouring in soybean and tomato. *International Journal of Agricultural Science*, **2**(1): 219-224

2. TONAPI VA, P HARINATH BABU, NA ANSARI, S VARANAVASIAPPAN, CH RAVINDER REDDY, SS NAVI AND N SEETHARAMA (2006b). Studies on development of seed colouring standards in paddy and maize. *Karnataka Journal of Agricultural Science*, **19**(2): 278-286

3. TONAPI VA, P HARINATH BABU, NA ANSARI, S VARANAVASIAPPAN, CH RAVINDER REDDY, SS NAVI AND N SEETHARAMA. 2006c. Studies on seed colouring in castor, sunflower, safflower. *Journal Oilseeds Research*, **23**(1): 72-80

4. HARINATH BABU P, VA TONAPI, NA ANSARI, S VARANAVASIAPPAN, CH RAVINDER REDDY, SS NAVI AND N SEETHARAMA (2007). Studies on seed colouring in red gram, black gram and Bengal gram. *Seed Research*, **35**(1): 58-65

5. NAVI SS, VK DESHPANDE AND SD KULLOLI (2006). Influence of seed colouring on rice weevil infestation during storability of hybrid sorghum. *Karnataka Journal of Agricultural Science*, **19**(2): 287-290

6. ABDUL-BAKI A AND JD ANDERSON (1973). Vigour determination in soybean seed by multiple criteria. *Crop Science*, **13**: 630-633.