

Studies On Seed Coloring In Soybean And Tomato

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ABSTRACT

The investigations on the effect of seed colouring on soybean and tomato seeds encompassing 25 dyes were conducted to identify non-deleterious and deleterious dyes based on their effect on seed quality. The dyes namely Rhodamine-B, Fast green and Malachite green for soybean and Rhodamine-B and Fast green for tomato are the best dyes for seed colouring at 0.75% concentration. In this paper we discuss developing colour standards for soybean and tomato seeds and their resultant implications for Indian seed industry.

Key words: seed colouring, soybean, tomato

INTRODUCTION

The history of seed colouring in the international arena suggest that colour standards in Canada, United States of America and other European countries were established as per policies regarding colouration of treated seeds and trade memorandums that were issued on July 13, 1967 Anonymous, (1967). In co-operation with CACA Technical Committee, Board of Grain Commissioners and other officials of the Canada department of agriculture, the plant products division has developed a colour standard for cereal seed treatment. This standard was established in order to facilitate treated seed in food or feed grain channels. As of January 1st, 1968 all cereal seed treatment products accepted for registration on renewal under the terms of the pest control products act must confirm to the colour standard. Products appearing on the market as a result of carry over stocks shall not be considered to be in violation of the requirement until January 1st, 1969. In line with this, Canada department of agriculture has given an outline of the laboratory method for the preparation of the standard to which all cereal seed treatment products must be compared when used according to label directions. In addition, in Canada, it is required at the time of submitting application for registration or renewal, a one half pound sample of the cereal seed which has been treated with the candidate product, using the attached procedure at

the dosage specified on the product label, must be submitted to the pesticide unit for examination.

Several different types of dyes have been used successfully for colouring seeds, including acids dyes, basic dyes, direct dyes and pigments. The basic dyes are used most frequently because of their strong, brilliant shades, which can provide distinctive colour inspite of the natural colouration of the seeds, and because of their economy, on an equal colour basis, versus other dye types. The dye is added to the seeds as solution or suspension and blended to give an even beverage. However, the quantity of the dye required at 0.75 per cent concentration (prepared by dissolving 0.25 g of dye in 16.50ml water plus 15.0ml of ethylene glycol) varies with the individual dyes used in seed colouring and kind of seed to which the colour is to be imparted. Several dyes have been approved by department of agriculture in Canada and United States of America, viz., Rhodamine-B, Tartrazine, FD and C blue, Methylene blue, Methyl violet 2B, D and C red, D and C violet, D and C green and pigment red, based on their non-toxic nature in regard to seed germinability Anonymous, (1979).

To be precise, processors colour seeds because it is required by law to avoid accidental use of treated seeds as food or feed. Some people colour seeds with a specific colour as a trademark, just to identify their seeds. But, to use such dyes, which in fact are chemical formulations, it is necessary to prove that they are non-toxic with respect to seed germination, vigour potential and viability; the information about which is not available to the extent it can be used in seed industry.

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But, till date there are very few and isolated studies in India to establish colour standards to pave the way for coloring the seeds by incorporating the provisions in the seed quality control and seed trade in India. Since the literature scan has indicated absence of any relevant literature in developing colour standards in India except the work of Tonapi (1988) and Tonapi and Karivaratharaju (1994) in sorghum, Vivekanandan (1997) and Basavaraj et al. (1999) in soybean. In other countries too, colour standards are propriety of individual private companies. To be precise, processors colour seeds because it is required by law to avoid accidental use of treated seeds as food or feed. Some companies colour seeds with a specific colour as a trademark just to identify their seeds. In this paper we discuss developing colour standards for soybean and tomato and their resultant implications for Indian seed industry.

MATERIALS AND METHODS

The investigations, on seed colouring were conducted with Soybean (Cv. JS 335) and Tomato (Cv. Pusa Ruby) encompassing 25 dyes namely Rhodamine-B, Cotton blue, Fuchsin, Neutral Red, Gentian Violet, Methylene blue, Crystal violet, Congored, Fast green, Bromocresol Purple, Phenol red, Nigrosine, Erichro black T, Ammonium purpureate (murexide), Bromocresol green, Malachite green, Methyl red, Methyl orange, Tital yellow, Indigo carmine along with commercially available natural dyes in the market namely Kumkum, Yellow, Pink, Blue and Brick red to develop and recommend color standards after assessing their effect on seed quality. The chemical composition of the dyes is given in Table 1.

Preparation of the dye :

All the dyes were prepared at 0.75% concentration by dissolving 0.25 gm of dye in 16.5 ml water and 15.0 ml ethylene glycol Tonapi, (1988).

Seed colouring procedure :

In order to obtain the desired dye intensity, individual dye solutions in specified quantities were added on to 10 g of seeds of each variety placed in a 1000 ml Erlenmayer flask, slowly down the sides of flask with a pipette. The flask was shaken for 3-5 minutes to give uniform coverage of individual dye to the seed. The seeds thus coloured were subjected for laboratory evaluation to assess the effect of these dyes on seed quality and field emergence as influenced by various biotic and abiotic factors.

Observations:

The effect of seed coloring dyes on seed germination, root and shoot length of seedlings. Dry weight and seedlings, speed of germination, field emergence and seed germination in exhaustion test, speed of germination, was recorded following International Seed Testing Association Standards Anonymous, (1985). Seed vigor was assessed through vigor index calculated as the product of root length

and seed germination and expressed as absolute value Abdul Baki and Anderson, (1973).

Electrical conductivity of seed leachate Presley, (1958), Seed germination in chemical soak test, Seed germination in D-manitol soak test Lad, (1986), α -amylase

Table 1: Chemical and natural dyes used in seed colouring studies

S.No.	Dye	Chemical composition
Chemical dyes		
1.	Indigo carmine	$C_{16}H_8N_2O_2 (SO_3Na)_2$
2.	Titan yellow	Dehydrothio-P-toluidine
3.	Methyl orange	$Me_2NC_6H_4N : NC_6H_4SO_3Na$
4.	Methyl Red	$Me_9NC_6H_4N : NC_6H_4COOH$
5.	Nigrosine	$C_{38}H_{27}N_3$
6.	Erichro Black-T	$C_{20}H_{12}N_3O_7Sna$
7.	Ammonium purpureate	(Murexide) $(NH_4)_4 P_2O_7$
8.	Bromocresol green	$C_{21}H_{14}Br_4O_5S$
9.	Bromocresol purple	$C_{19}H_{10}Br_2Cl_2O_2S$
10.	Crystal violet	$C_{25}H_{30}ClN_3$
11.	Malachite green	$Me_2NC_6H_4C_6H_5C : NC_6H_4Me_2Cl$
12.	Congo red	$C_{32}H_{22}O_6N_6S_2Na_2$
13.	Phenol Red	$C_{19}H_{14}O_5S$
14.	Cotton Blue	$C_{32}H_{25}N_3O_9S_3Na_2$
15.	Gentian violet	$C_{25}H_{30}ClN_3$
16.	Fuchsin	$C_{20}H_{17}N_3Na_2O_9S_3$
17.	Methylene Blue	$C_{19}H_{10}Br_2Cl_2O_2S$
18.	Rhodamine-B	$C_{28}H_{31}Cl N_2O_3$
19.	Neutral red	$Me_2NC_6H_4N : NC_6H_4MeNH_2.HCl$
20.	Fast green	$C_{37}H_{34}N_2Na_2O_{10}S_3$
Natural dyes		
21.	Natural dye - Kum kum	
22.	Natural dye - Brick Red	
23.	Natural dye - Blue	
24.	Natural dye - Yellow	
25.	Natural dye - Pink	
26.	Control	

activity. Simpson and Naylor, (1962) and Dehydrogenase activity Kittcock and Law, (1968) were evaluated to assess the effect of dyes on biochemical composition of seeds. All the tests were conducted in four replications consisting 400 seeds. Fisher's method of analysis of variance was applied for data analysis. Critical differences were calculated at $P = 0.05$.

RESULTS AND DISCUSSION

Influence of seed colouring on germinative, physiological and biochemical indices of seeds in soybean cv. JS-335 (Table 2) (Fig. 1)

The seed colouring studies were conducted in soybean. In this, the various seed colouring dyes involved

Table 2: Influence of seed colouring on seed germination (%), seedling growth and vigour index in soybean (Cv. JS - 335)

S. No	Name of the dye	Seed germination (%)	Root length (cm)	Shoot length (cm)	Whole seedling length (cm)	Dry weight of seedling (g)	Vigour index (RL x G%)	Speed of germination	E.C (μ mhos/cm)
1.	Rhodamine-B	65 (53)#	7.83*	14.10	21.93*	0.183*	515*	4.7	410
2.	Cotton Blue	45*(42)	6.76*	11.43	18.20	0.110*	307	4.5*	411
3.	Fuch sine	40*(39)	2.40	7.53	9.93	0.153*	99*	4.0*	412
4.	Neutral Red	26*(30)	3.77	10.07	13.84	0.053	97*	2.5*	410
5.	Gentian violet	45*(42)	4.33	12.47	16.80	0.213*	189	4.2*	415*
6.	Methylene Blue	44*(41)	7.77*	12.30	20.07	0.233*	355	4.3*	418*
7.	Crystal violet	46*(42)	3.77	9.37	13.13	0.163*	174	4.0*	410
8.	Congo red	32*(34)	4.40	7.53	11.93	0.080	140	3.1*	412
9.	Fast green	54*(47)	8.27*	12.43	20.70	0.153*	451*	4.7*	412
10.	Bromocresol purple	49*(44)	4.93	9.03	14.30	0.200*	251	4.9	413
11.	Phenol Red	25*(29)	1.87	9.47	11.33	0.127*	49*	2.7*	420*
12.	Nigrosine	25*(29)	4.57	8.47	13.03	0.153*	120	2.9*	410
13.	Erichro black-T	30*(33)	1.80	7.07	8.87	0.060	55*	3.2*	420*
14.	Mureoxide (Amm.pur)	28*(32)	4.50	5.53*	10.03	0.107*	152	2.7*	412
15.	Bromocresol green	29*(31)	1.40	7.67	9.07	0.053	35*	4.6*	411
16.	Malachite green	31*(34)	3.50	10.07	13.57	0.083	110*	3.5*	411
17.	Methyl Red	29*(33)	5.06	11.63	16.70	0.100*	150	2.5*	430*
18.	Methyl Orange	40*(39)	6.40*	9.73	16.13	0.187*	266	4.8	429*
19.	Titan yellow	21*(27)	4.07	5.07*	9.14	0.017	102*	2.5*	412
20.	Indigo caramine	44*(41)	4.67	9.97	14.63	0.09	214	3.8*	412
21.	Natural dye -kumkum	27*(31)	2.17	7.47	9.63	0.130*	56*	2.3*	426*
22.	Natural dye - yellow	9*(14)	1.67	6.00*	7.67*	0.037	22*	2.2*	429*
23.	Natural dye - pink	18*(21)	2.60	8.57	11.17	0.027	72*	2.5*	431*
24.	Natural dye - Blue	38*(37)	7.00	12.73	19.73	0.193*	279	3.4*	427*
25.	Natural dye -Brickred	31*(33)	4.40	9.33	13.73	0.163*	139	3.1*	430*
26.	Control	74 (59)	3.50	11.17	14.67	0.009	258	5.0	410
	S.E.d±	7.287	1.162	2.235	3.043	0.038	70.26	0.14	1.585
	C.D (0.05) *	15.157	2.418	4.650	6.330	0.077	146.15	0.28	3.193

#Figures in parentheses are arcsine transformed values.

Developing colour standards also exhibited both positive and deleterious influences on seed quality parameters in the range viz., seed germination (74 - 9%), root length (8.27 - 1.40 cm), shoot length (14.1 - 5.07 cm), whole seedling length (21.9 - 8.87 cm), dry weight of seedlings (0.233 - 0.009 g), vigour index (515 - 22), field emergence potential (45.6 - 8.3%), and speed of germination (5 - 22).

The biochemical and enzyme activity including performance under stress had wide ranging influences in terms of membrane integrity as indicated by electrical conductivity (410 - 431 μ mhos/cm), α-amylase activity (3.3 - 2.7 mm), dehydrogenase activity (0.280 - 0.240 OD). The range of seed germination as influenced by seed colouring in soybean under stress tests viz., were in range of 69 - 25%, 65 - 20% and 66 - 22% in chemical soak test, exhaustion test and D-mannitol soak test respectively.

The detailed analysis of the data could conveniently help to categorise all the 25 dyes into best, mid range and most deleterious in relation to their effect on seed quality. The dyes namely Rhodamine-B, Fast green and

Malachite green are the best and most favoured dyes in view of their promoting effect on seed quality in soybean. The most deleterious dyes in soybean were identified as the natural dyes yellow and pink others were in mid range.

Based on vibrant colours, shine, hues and uniform colouration imparted on soybean seed as compiled from the survey of seed growers, processors and seed industry people out of total sample of 100, it could be brought out that most favoured seed colouring dyes out of the best dyes already enlisted on the basis of visual colour index are Rhodamine-B, Fast green and Malachite green.

Influence of seed colouring on germinative, physiological and biochemical indices of seeds in tomato cv. Pusa Ruby (Table 3) (Fig. 2).

In tomato, the various dyes had promoting as well as deleterious impact significantly on seed germination (69 - 0 %), root length (9.0 - nil cm), shoot length (13.2 - nil cm), whole seedling length (21.93 - nil cm), dry weight of seedlings (0.017 - nil g), vigour index (416 - nil), field emergence (57.6 - nil %) and speed of germination (6.6 - nil).

The biochemical and enzyme activity including

dyes, which in fact are chemical formulations, it is necessary to prove that they are non-toxic with respect to seed germination, vigour potential and viability; the information about which is not available to the extent it can be used in seed industry.

However, the promotory effect of some of the dyes may be due to the probable stimulatory effect on enzymes like α -amylase and dehydrogenase activity and their release during seed germination, because of which the faster rate of growth of seedlings becomes evident, as seen in the present study; in the form of higher root length, shoot length, and maximum dry weight of seedlings, including vigour index. The inhibitory toxic effect of Natural dyes Kumkum, Blue, Pink, Yellow, Brick red and Methyl red indicated the entry of the dye, though in very small quantity, into the seed, due to which probably the active chemical ingredient groups of each of these deleterious dyes might have interfered with seed energetics, enzyme release and macromolecule degradation in seed during seed germination to result in the form of maximum number of abnormal seedlings, lower vigour and decreased performance under stress conditions as evaluated through exhaustion test.

Through this study on colour standards, we would like to propose for efforts to standardize reproducible colour standards for crops as in USA, Canada and Europe. We propose that this provision can also be incorporated under the regulations of seed quality and pest control act after suitable modifications with the text that "Where the physical properties of the control product are such that the presence of the control product may not be recognized when used and is likely to expose a person or domestic animal to severe health risk, the control product shall therefore be denaturized by means of colour, odour or such other means as the central seed committee may approve to provide a signal or warning as to its presence". Where the seed is packaged, the package should bear a label with words "the seed is treated and colored with" followed by the name of the control measure product and the seed coloring dye, including the common name or chemical name of its active ingredient together with appropriate precautionary symbol and signal or warning words as the seed testing Committee approves. If the treated-colored seed is sold and shipped in bulk, the shipping documents should bear information setting forth the common name or chemical name of active ingredient of both chemical and the seed coloring dye with a sub note that "seed coloring dye used is not injurious to seed". This will enable seed industry to adopt individual colors as their trademark including propriety coloring of parental lines to identify their seeds and seed coloring may substantially aid in preventing accidental usage of treated seeds as food or feed, or may help in upgrading the visual quality of blonded (discolored

and rain soaked) and blended seeds (but, still maintaining seed germination at (75%) and above certification standards under emergent situations where there is a scarcity of seeds.

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