Indian Journal of Agricultural Sciences **92** (6): 741–6, June 2022/Article
https://doi.org/10.56093/ijas.v92i6.113176

Effect of seed discolouration on seed quality parameters in paddy (*Oryza sativa*)

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Received: 27 July 2021; Accepted: 10 March 2022

ABSTRACT

The production and productivity of paddy (*Oryza sativa* L.) has been reported to be severely affected by an emerging disease called seed discolouration. It is a complex disease because several pathogenic fungi infect rice grains in the field. The infection reduces the seed quality by producing harmful mycotoxins that are hazardous to humans and animals. The current study was carried out from 2016–19 at IARI, New Delhi to determine the effect of seed discolouration on seed quality parameters in terms of physio-chemical changes. The findings revealed a significant loss in the seed quality of paddy in popular rice varieties. We observed a significant reduction in the germination percentage (9–42%), seedling length (3.4–33.3%), seedling dry weight (2.7–36.5%), and seedling vigour index I (20.1–52.5%) and II (12.3–53.7%), whereas an increase in electrical conductivity (EC) (18.7–38.9%) were observed in discoloured seeds compared to healthy seeds. Similarly, the significant decrease in starch (5.3–37.6%) and protein (1.6–46.2%) content was recorded, whereas, an increase in total antioxidant (3.3–37.1%) and total phenol content (0.58–26.03%) was seen in discoloured seeds, as compared to healthy seeds. The results show that the seed quality is highly compromised in discoloured seeds, as compared to healthy seeds and need to be addressed through the development of different management practices.

Keywords: Biochemical parameters, Paddy, Seed discolouration, Seed quality parameters

Rice (*Oryza sativa* L.) is one of the most commonly consumed cereals in India, accounting for more than 60% of the population (Adam *et al*. 2018). It is an important food crop that provides food coverage for more than two-thirds of the country's population and a source of income for millions of rural households. Since it is cultivated in different ecologies and climatic conditions, the crop is subjected to a variety of stresses, including biotic and abiotic stresses (Wilson and Talbot 2009). Among the biotic stresses, brown spot, bacterial blight, blast, sheath blight and tungro have become more serious, and many minor diseases like false smut, bakanae, early seedling blight, sheath rot, and seed discolouration have emerged as major problems in rice cultivation (Raghu *et al*. 2018).

Seed discolouration, also known as dirty panicle disease, is one of the emerging minor disease that is causing a serious problem in rice growing areas around the world (Narain 1992). The disease is widespread in Asia, Africa and Latin America causing significant damage to the yield and quality. It also serves as a visible indicator of seeds having lower quality in association with microorganisms (Chandramani and Awadhiya 2014). The minimum threshold for the procurement of discoloured paddy seeds is set at 3%, and all samples above that are rejected. Seed discolouration is a term for the change in colour of mature seeds from their initial colour, and seed has several issues in the seed certification programme. It significantly reduces seed quality due to toxin accumulation and leads to loss of seed quality, nutrients and yield thereby reducing the economical and marketable value of the crop. The loss due to seed discolouration in rice was estimated to be between 20–25% (Ghose *et al.* 1960). In this context, present study was undertaken to determine the effect of seed discolouration of paddy on seed quality parameters. This study gives us a holistic idea of changes in the biochemical composition and seed quality parameters.

MATERIALS AND METHODS

Survey and sample collection of discoloured seeds: Twenty-five varieties of paddy seeds were collected from the paddy fields of Indian Agricultural Research Institute during the *Kharif* of 2016–19. The samples were brought to the Division of Seed Science and Technology, ICAR-IARI, New Delhi (28.6377° N, 77.1571° E) and were sorted out into healthy and discoloured seeds based on the morphology of seeds for further analysis.

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Seed quality analysis: Both healthy and discoloured seeds were subjected to standard germination test and the vigour and viability were evaluated by using ISTA rules, 2019. The germinability of seed was determined by using between paper methods.

Standard germination test: Four hundred seeds were used for conducting a standard germination test. With the help of a counting board, hundred seeds for each replicate were placed between two moist germination papers. Then the germination papers were folded and rolled up carefully along one edge ensuring that no excess pressure is placed on the seeds. These were wrapped in wax paper to prevent surface evaporation and placed in an upright position in a germinator at 25°C temperatures. After 5 days of incubation, the seedlings were examined for normal, abnormal seedlings, fresh ungerminated and dead seeds following the International Rules for Seed Testing (2019). A second count of abnormal seedlings was made after the completion of 14 days. However, the germination percentage was recorded based on normal seedlings only. Seedling length and seed dry weight were recorded from which seedling vigour index I and II respectively was calculated as suggested by (Abdul Baki and Anderson 1973).

Electrical conductivity: Four sub-samples of 50 seeds were weighed (0.001 gm accuracy) and placed in plastic cups containing 75 ml deionized water in a BOD-type germination chamber at 25°C for 24 h. The electrical conductivity of the soaking solution was measured after this period, and the findings were represented in µS/cm/g of seed (AOSA 1983).

Biochemical analysis: Estimation of starch was done using Clegg's technique (1956). Soluble protein was estimated using the technique provided by Bradford (1976). Total antioxidants were calculated using Benzie and Strain's techniques (1999). Total phenol estimation was done according to Single to and Rossi (1965). These all are the standard procedures for the biochemical analysis.

Statistical Analysis: The data on quantity observations recorded were subjected to statistical analysis by adopting a complete block design using OPSTAT and the percentage data were transformed into arcsine value for analysis.

RESULTS AND DISCUSSION

Over the last few decades, rice output and productivity have grown by many folds. Inventions in rice research after the green revolution witnessed an increase in production. The concern of biotic and abiotic stresses has been raised along with an increase in rice productivity. Biotic stresses become a major hurdle for further improvement in yields and due to changes in pathogen patterns and production techniques, the major disease continues to inflict much more damage. On the other hand, as the climate changes, many minor diseases that were previously less important have become major problems (Raghu *et al*. 2018). Seed discolouration is one such disease that is becoming a serious issue in rice cultivation (Arshad *et al*. 2009). Seed discolouration is caused by a number of organisms, including pathogens and saprophytes, making it a complex problem. Significant decreases in grain size, nutrient content and yield are the important factors affected by seed discolouration (Chhabra and Vij 2020).

Effect on seed quality parameter: Effect of seed discolouration on seed quality parameters like germination, seedling length, seedling dry weight, vigour index I and II and Electrical conductivity (EC) both in healthy and discoloured seeds were estimated (Table 1). The results showed that discoloured seeds had a significant reduction in the quality parameters as compared to healthy seeds (Fig 1). The maximum loss in germination percentage was observed in Kudrat-2 (41.8%) followed by IR 79156B (34.8%). The minimum loss in germination percentage was recorded in IR 580559A (8.6%). Similarly, the maximum reduction in the seedling length was observed in Pusa 2-21 (33.3%), seedling vigour index–I in Pusa 2-21 (52.5%), seedling dry weight in APMS 6B (36.46 %), seedling vigour index II in Kudrat-2 (53.7%) and least reduction in seedling length was observed in Dudeshwar (3.4%), seedling vigour index–I in IR 580559A (20.1%), seedling dry weight in Vasumati

Healthy seedlings Discoloured seedlings

Fig 1 Germinated seedlings of healthy and discoloured seeds.

Table 1 Seed quality parameters of healthy and discoloured seed (values in parenthesis are arcsine transformed values)

Variety	Germination (%)		Seedling length (cm)		Seedling vigour index (SVI–I)		Seedling dry weight (mg)		Seedling vigour index (SVI–II)		Electrical conductivity (µS/cm/gm)	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Vijetha	92(73.96)	72(58.08)	20.12	17.80	1856.01	1281.43	66.4	55.6	6.13	4.01	33.06	42.96
Komal	92(73.37)	76(60.36)	18.90	16.04	1733.77	1211.02	58.9	47.2	5.40	3.56	35.28	48.04
Pusa Sugandha-5	87(68.89)	59(50.17)	21.01	17.57	1827.96	1036.83	65.9	57.5	5.73	3.39	31.85	40.69
Pusa 2-21	96(78.11)	68(55.56)	29.69	19.79	2835.40	1345.49	83.1	72.6	7.93	4.93	30.00	49.07
Dudeshwar	93(75.04)	74(59.20)	24.13	23.30	2250.43	1718.62	73.3	66.5	6.84	4.90	30.28	43.69
Vikramarya	84(66.06)	74(59.36)	25.95	19.86	2166.69	1469.64	99.9	71.7	8.34	5.30	30.04	41.24
Pusa-33	88(69.63)	71(57.13)	17.83	16.54	1564.58	1166.07	61.6	52.1	5.40	3.67	30.11	40.18
IR-64608 B	94(75.53)	72(57.73)	20.82	17.65	1946.67	1261.74	79.5	68.6	7.44	4.90	35.38	48.77
PR113	91(72.70)	73(58.69)	23.79	21.04	2164.89	1535.68	97.6	65.8	8.88	4.80	29.25	42.08
Pusa 6A	84(66.47)	67(54.63)	21.40	18.13	1797.60	1205.87	68.6	52.5	5.77	3.49	36.03	49.02
IR 580559B	93(74.52)	75(59.68)	23.75	18.86	2202.50	1404.82	80.9	65.1	7.50	4.85	35.49	46.39
APMS 6B	92(73.91)	78(62.05)	19.09	15.36	1761.05	1198.34	67.2	42.7	6.20	3.33	33.06	40.68
Satabdi	91(72.17)	78(61.78)	26.11	21.23	2362.96	1645.33	76.1	62.5	6.89	4.84	30.12	48.09
IR-64608B	90(71.40)	70(56.96)	22.98	19.34	2062.75	1358.64	64.0	56.6	5.74	3.97	35.93	46.31
IR 8	88(70.07)	70(56.62)	24.75	19.63	2183.89	1369.43	58.3	48.6	5.14	3.39	34.25	45.06
IR 6897A	84(66.11)	64(52.98)	22.83	20.26	1906.31	1291.36	82.7	68.5	6.90	4.37	31.21	47.28
IR 79156B	82(65.29)	54(47.14)	25.22	18.88	2078.17	1014.98	69.8	58.0	5.75	3.12	28.75	43.82
Vasumati	84(66.05)	71(57.58)	30.40	22.08	2538.39	1573.44	65.0	66.8	5.43	4.76	29.36	42.80
IR 68902B	95(76.87)	80(63.30)	28.61	23.89	2710.80	1905.23	72.3	63.9	6.85	5.10	33.21	45.81
Pyari	92(73.58)	73(58.57)	26.36	17.81	2418.22	1295.68	57.4	43.2	5.26	3.14	28.77	41.96
IR 79156A	80(63.09)	63(58.57)	27.21	25.90	2162.93	1631.91	84.2	68.1	6.69	4.29	32.50	51.30
Kudrat-2	86(67.69)	50(44.84)	20.69	18.66	1769.00	928.34	57.3	45.6	4.90	2.27	36.45	45.69
IR 68897B	94(75.91)	69(56.01)	23.60	18.91	2218.71	1300.06	68.8	59.1	6.47	4.07	31.21	44.25
IR 580559A	87(68.93)	80(63.15)	19.76	17.27	1719.12	1372.97	69.3	59.2	6.03	4.71	30.04	47.86
Pusa-44	93(74.67)	63(52.67)	23.56	20.89	2190.77	1321.50	68.1	56.6	6.34	3.58	29.08	44.69

	Disease incidence (A)	Varieties (B)	A*B	Disease incidence (A)	Varieties (B)	A*B	Disease incidence (A)	Varieties (B)	A*B	Disease incidence (A)	Varieties (B)	A*B	Disease incidence (A)	Varieties (B)	A*B	Disease incidence (A)	Varieties (B)	A*B
CD @5%	0.889	3.144	4.446	0.189	0.667	.0.944	15.791	55.830	78.956	2.11	7.45	10.53	0.049	0.175	0.247	0.380	1.342	1.898
SE(M)	0.318	1.124	1.589	0.067	0.238	0.336	5.62	19.868	28.098	1.06	3.75	5.30	0.018	0.062	0.088	0.133	0.471	0.666

Table 2 Biochemical composition of healthy and discoloured seed

Variety	Protein Content (mg/gm)		Starch (mg/gm)		Total phenol (mg GAE/100g)		Total antioxidant (Fe^{2+}µM/g dry weight)	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Vijetha	4.71	3.43	82.87	67.94	49.70	55.54	4.03	5.53
Komal	4.19	3.81	77.43	50.77	42.29	57.17	7.74	8.09
Pusa Sugandha-5	4.13	3.74	81.00	52.81	43.92	48.82	6.61	8.29
Pusa 2-21	4.66	4.04	81.51	60.15	48.13	55.23	5.99	6.76
Dudeshwar	4.77	4.49	86.60	61.64	46.45	54.49	4.71	5.53
Vikramarya	4.64	4.09	88.64	61.98	49.34	54.46	6.99	8.31
Pusa-33	4.98	4.82	81.34	50.77	47.17	53.37	3.81	4.59
IR-64608 B	4.77	4.39	82.49	54.54	49.05	55.12	7.32	7.95
PR113	4.80	4.26	83.04	78.62	45.18	59.28	7.16	8.18
Pusa 6A	5.17	4.84	80.32	70.47	58.22	69.22	7.55	8.24
IR 580559B	5.46	5.02	80.66	50.77	59.04	61.14	5.39	6.38
APMS 6B	4.75	3.98	86.94	59.94	52.53	58.98	7.04	8.86
Satabdi	4.31	3.77	78.96	55.87	63.43	72.59	3.43	5.45
IR-64608B	4.74	4.39	84.06	69.62	47.26	52.38	6.17	7.07
IR 8	4.79	4.28	81.85	63.85	60.72	66.90	6.75	7.23
IR 6897A	4.75	4.6	81.68	70.98	47.14	51.20	3.82	5.55
IR 79156B	4.97	4.62	82.53	60.45	55.73	64.71	7.87	8.14
Vasumati	4.69	4.06	82.02	61.13	59.16	65.78	5.68	7.41
IR 688902B	4.75	4.50	80.32	60.45	46.72	52.57	7.31	7.91
Pyari	5.09	4.76	80.66	53.15	51.51	51.81	4.68	5.28
IR 79156A	4.86	4.78	88.12	66.74	49.10	53.73	7.16	8.55
Kudrat-2	5.22	4.89	84.91	67.75	55.90	62.11	6.96	7.68
IR 68897B	5.20	2.80	85.06	62.24	49.87	56.01	6.54	7.41
IR 580559A	5.02	4.76	84.57	71.49	52.05	56.08	6.20	7.46
Pusa-44	4.61	4.27	83.56	61.08	52.47	57.89	7.50	8.36

	Disease incidence (A)	Varieties (B)	A*B	Disease incidence (A)	Varieties (B)	A*B	Disease incidence (A)	Varieties (B)	A*B	Disease incidence (A)	Varieties (B)	A*B
CD @5%	0.049	0.174	0.246	0.647	2.288	3.236	0.59	2.085	2.949	0.062	0.218	0.308
SE(M)	0.017	0.061	0.086	0.23	0.814	1.152	0.207	0.732	1.035	0.022	0.077	0.11

(2.7%) and seedling vigour index-II in Vasumati (12.3%). Maximum increase in leachates (EC) was seen in the case of Pusa 2-21 (38.9%) and a minimum increase in leachates was observed in APMS 6B (18.7%). Our observations indicated that healthy seeds performed better, as compared to diseased seeds in terms of seed quality parameters. Loss in performance of seed quality parameters in discoloured seeds is due to pathogen infection and that makes the seeds vulnerable to seed deterioration and ultimately causes reduced germination and poor crop establishment conditions. This may be due to mycelia coating on seeds or secretion of some toxins which cause inhibition of germination due to damage of embryo of seeds. Sharma *et al*. (1993), Neerguard (1977), and Bag *et al*. (2010) also reported the involvement of seed discolouration causing micro agents in inhibiting germination of seed. The seed discolouration adversely affected the seed germination, seedling length and seedling dry weight. The decrease in seed germination, seedling vigour index I and II and increase in Electrical conductivity due to seed discolouration in different cultivars were also reported by Zulfiki *et al*. (1991) and Reddy *et al*. (2000).

Effect on biochemical composition: Effect of seed discolouration on biochemical parameters like starch, protein, total phenol and total antioxidant content was determined in both healthy and discoloured seeds (Table 2). The results indicated that a significant reduction in starch and protein content was recorded in discoloured seeds compared to healthy seeds. We observed contrasting trends in total phenol and total antioxidant content in discoloured seeds. The maximum reduction in starch content was recorded in Pusa-33 (37.6%) and protein content in IR 6897B (46.2%). Similarly, a minimum reduction in starch content and protein content was observed in PR113 (5.3%) and IR 79156A (1.6%), respectively. Total antioxidant and total phenol content were maximum in the case of variety Komal (26.0%) and total antioxidant content was maximum in Satabdi (37.1%). A minimum increase in total antioxidant content was observed in IR79156B (3.3%) and total phenol content in Pyari (0.6%). The decrease in starch content reported in the present study might be due to the rapid breakdown of starch by pathogen produced enzymes, or partial utilization of starch product or both (Mirocha and Zaki 1966). Duraiswamy and Mariappan (1983), and Sachan and Agarwal (1995) observed a decrease in starch content of paddy seeds due to seed discolouration. In the present study, significant changes in protein content were noticed in the discoloured seeds (Table 2). The seeds in IR 6897B (46.2%) recorded a maximum reduction of protein while the least reduction was seen in IR 79156A (1.6%) cultivar. Initially, the loss in protein content of discoloured seed may have been attributed to the hydrolysis of simple forms of proteolytic enzymes (Jamaluddin *et al*. 1977). Reddy *et al*. (2000) also observed that the reduction of protein content in the range of 29.7–36.7% in rice seeds is due to *S. oryzae* infection. However, Sachan and Agarwal (1995); Sumangala *et al*. (2009) discovered that seeds with discolouration had a substantial increase in protein content. The outcome of the present study showed a significant increase in phenolic content in discoloured seeds. An increase in phenolase activity in the host tissue, especially at and around infection sites, is a response seen in many diseases. A higher level of phenolics might suggest that the host's defence system has been activated, or that the pathogen has triggered a fast rate of phenolic synthesis, attributed to tissue necrosis. This finding was in agreement with Duraiswamy and Mariappan (1983) in seed discolouration and Gopalakrishnan *et al*. (2010) in the sheath rot of paddy.

From the observations, it is concluded that starch and protein may act as a source of nutrients for the fungus. An increase in total phenol and total antioxidants content in discoloured seeds might be due to the response of the host to the invasion fungi (Gopalakrishnan *et al.* 2010). These results clearly indicate that the quality reduction in discoloured seeds is mainly due to the activities of seed discolouration causing fungus.

The noteworthy information produced would be helpful in analysing the effect of paddy seed discolouration on seed quality and the biochemical composition of seeds. Seed discolouration had a significant ($P<0.05$) effect on seed quality parameters and biochemical composition of seeds. The germination percentage, seedling length, seedling dry weight, seedling vigour index I and II, starch and protein content were increased, while electrical conductivity, total antioxidant and total phenol content were increased in discoloured seeds.

ACKNOWLEDGEMENT

The authors are thankful to SERB for funding the research.

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