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Inter- and intra-plant variation in phenol content in grains of rice (*Oriza sativa* varieties using phenol colour reaction

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The phenol color test, which is an index of polyphenol oxidase activity, is a simple method for grouping the rice (*Oriza sativa* L.) varieties (Oka 1958, Abrol and Uprety 1972, Sivasubramanian and Ramakrishnan 1974, Vanangamudi *et al*. 1988). During phenol color test, phenol gets oxidized into dark color melanin pigment catalyzed primarily by the enzyme tyrosinase present on the seed coat and is under simple genetic control (Joshi and Banerjee 1970 and Bhowal *et al*. 1969). The phenol color reaction basically depends on the quality and amount of the enzyme which is present in the seeds (Walls 1965). Observation was made on the role of the phenolic compound present in seed which were suggested by the Joshi and Banerjee (1970). While working with rice cultivars with positive color reaction with phenol it was suggested that phenol oxidation is accomplished by two chemical reactions in the living organ. In the first reaction the aromatic ring of phenol can be further hydroxylated, and in the second reaction catechol or quinols can be oxidized to corresponding quinines (Harborne 1964 and Conn 1964). It was reported that the range of color intensity among germplasm accessions were different that might be due to variation in enzymatic activity, temperature, light, aeration and genetic background (Sivasubramanian and Ramakrishnan 1974, Kumar *et al*. 2017).

The following rice varieties belonging to different phenol colour reaction groups have been used in the study. The varieties were grown in *kharif* 2019. The mature seeds were harvested in different stages of flowering and grain maturity and panicles of plants separately and stored in ambient room conditions.

Black	Dark brown	Brown	Light brown	No colour
Krishnaveni	MTU1010	Satabdi	Falguna	Vandana
CSR 13	PNR 519	PD 4	Mahamaya	Satyabhama
CRD 300	Kranti	Jaya	IR-64	VL Dhan 206
PNR 381	Swarna	PR113	ADT 37	Triguna

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Fully matured and dried seeds were taken for the estimation of total phenol. Using palm de-husker, husk and kernel were separated. Kernel and husk were grounded separately and fine powder was made. It was kept in air tight container with liquid Nitrogen (-196˚c) until used for further procedure. Kernel/husk powder was used for phenol content estimation following the procedure suggested by Singleton *et al.* (1965).

Phenol content in husk at different stages of growth period was estimated. It was observed that mean of black colour variety 7.50 mg/g in case of CRD 300 and mean of no colour group was 5.25 in case of Vandana. Among 20 varieties mean of stage 1 was 6.88 mg/g phenol content, whereas in stage 2, 3 and 4 were 7.21, 6.25, and 5.92 mg/g phenol content, respectively (Table 1). Higher average phenol content was measured at second stage.

Phenol content was analyzed in the seeds of panicles of, viz. early tiller, mid tiller and late tiller (named as panicle 1, panicle 2 and panicle 3, respectively, collected at maturity stage showed that there was no significant difference among the panicles of the same plant in all the colour group varieties. Mean phenol content of the black colour Krishnaveni was 6.737 mg/g and that of no colour Satyabhama was 4.618 mg/g. Among 20 varieties panicle 1 showed 5.912 mg/g, panicle 2 had 5.912 and panicle 3 showed 5.909 mg/g (Table 2).

An analysis in the grain positioned in the top, middle and bottom portion in a panicle at grain maturity stage of 10 varieties in different colour reaction groups showed that there was a significant difference in the phenol content in the grains positioned at top, middle and basal portion of the panicle. In general, the seeds at the middle of the panicle showed the highest mean phenol content. In maturity stage of the grains the highest mean phenol content of 5.952 mg/g was found in the middle portion and the lowest mean of 5.66 mg/g in the bottom portion (Table 3).

Phenol content were studied at different stages of maturity and growth which showed higher amount of phenol content in case of pre-anthesis which is immature stage of development. In this stage 12 isozymes of PPO have been reported in wheat (Kruger 1976). PPO activity was high at

Table 1 Total phenol content (mg/g) at different stages during growth period in husk of the paddy varieties

Colour group	Variety	Total phenol content (mg/g)				
		Stage 1	Stage 2	Stage 3	Stage 4	Mean
Black	Krishna Veni	7.98	8.15	7.06	6.77	7.49
	CSR 13	7.90	8.20	7.01	6.82	7.48
	CRD 300	7.99	8.20	7.07	6.74	7.50
	PNR 381	7.86	8.11	6.99	6.66	7.41
Dark brown	MTU1010	7.58	7.80	6.70	6.46	7.14
	PNR 519	7.71	7.98	6.77	6.58	7.26
	Kranti	7.47	7.85	6.74	6.58	7.16
	Swarna	7.63	7.86	6.76	6.42	7.17
Brown	Satabdi	6.65	7.29	6.52	6.01	6.62
	PD 4	6.78	7.28	6.48	6.13	6.67
	Jaya	6.78	7.18	6.49	6.10	6.64
	PR113	6.67	7.32	6.36	6.19	6.64
Light brown	Falguna	6.53	6.79	6.07	5.61	6.25
	Mahamaya	6.35	6.61	5.92	5.53	6.10
	IR-64	6.47	6.66	6.15	5.60	6.22
	ADT 37	6.34	6.69	6.06	5.69	6.19
No colour	Vandana	5.68	5.93	4.88	4.52	5.25
	Satyabhama	5.70	6.11	4.99	4.68	5.37
	VL Dhan 206	5.79	6.22	5.06	4.62	5.42
	Triguna	5.67	5.90	5.00	4.78	5.34
Mean		6.88	7.21	6.25	5.92	
CD (P=0.05): Var:0.027; stages:0.059; Var x stages :0.119						

Stage1: pre-anthesis; stage 2: 10 days after anthesis (milk); stage 3: 20 days after anthesis (dough) and stage 4: 30 days after anthesis (harvest maturity).

Table 2 Variation in phenol content (mg/g) in different panicles of a plant in the varieties of phenol colour groups

Colour group	Variety	Phenol content (mg/g)			
		Panicle 1	Panicle 2	Panicle 3	Mean
Black	Krishna Veni	6.736	6.736	6.739	6.737
	CSR 13	6.774	6.774	6.772	6.773
	CRD 300	6.726	6.726	6.718	6.723
	PNR 381	6.71	6.71	6.718	6.712
Dark brown	MTU1010	6.549	6.549	6.555	6.551
	PNR 519	6.57	6.57	6.586	6.576
	Kranti	6.49	6.49	6.489	6.49
	Swarna	6.41	6.41	6.402	6.407
Brown	Satabdi	6.126	6.126	6.116	6.122
	PD 4	6.051	6.051	6.037	6.046
	Jaya	6.056	6.056	6.059	6.057
	PR113	6.12	6.12	6.122	6.121
Light brown	Falguna	5.606	5.606	5.553	5.589
	Mahamaya	5.199	5.199	5.2	5.2
	IR-64	5.296	5.296	5.289	5.293
	ADT 37	5.788	5.788	5.786	5.788
No colour	Vandana	4.691	4.691	4.684	4.689
	Satyabhama	4.616	4.616	4.623	4.618
	VL Dhan 206	4.819	4.819	4.817	4.818
	Triguna	4.9	4.9	4.908	4.902
	Mean	5.912	5.912	5.909	
CD (P=0.05): Var: 0.040; Panicle: NS;Var.×Panicle: NS					

Table 3 Variation in phenol content (mg/g) in grains borne in top, middle and basal position of panicle at maturity in the selected varieties

Variety	Phenol content(mg/g) in grains at			Mean
	Top	Middle	Basal	
CSR 13	6.767	6.836	6.385	6.663
CRD 300	6.729	6.763	6.456	6.649
Kranti	6.495	6.549	6.347	6.464
PNR 519	6.56	6.449	6.197	6.402
Jaya	6.056	6.001	5.46	5.839
PR113	6.115	6.181	5.744	6.013
Falguna	5.692	5.639	5.646	5.659
Mahamaya	5.649	5.556	5.48	5.562
Satyabhama	4.666	4.725	4.585	4.659
Vandana	4.632	4.825	4.314	4.59
Mean	5.936	5.952	5.661	
CD (P=0.05)Var: 0.073;Position: 0.040;V×P: 0.126				

early stages of wheat kernel development and decreases with kernel maturation (Taneja *et al*. 1974 and Kruger 1976). A large part of PPO activity was localized in the endosperm at 21–25 days after anthesis (Kruger 1976).

In plants, PPOs are localized in plastids and their phenolic substrates are mainly located in the vacuole so that enzymatic browning occurs only when this subcellular compartmentation is lost (Vaughn *et al.* 1988**).** PPOs are bi-copper metalloenzymes and possess two conserved copper-binding domains, CuA and CuB, responsible for copper coordination and interaction with molecular oxygen and phenolic substrates (Steffens *et al.* 1994**).** The existence of phenol content at pre anthesis stage could possibly be due to this fact. However, no colour reaction in any of the varieties at pre-anthesis stage could be due to absence or very low amount of Tyrosinase enzyme present at this stage. This needs to be further studied.

Polyphenol oxidase is the key of phenol color reaction which occur due to tyrosinase, L-Dopa, catechol and other small intermediates. Polyphenol oxidases (PPOs) are involved in the time-dependent darkening and discolorations of paddy seed glumes. Polyphenol oxidases catalyze the formation of quinones from phenols in the presence of molecular oxygen. The quinones react with amines and thiol groups or undergo self-polymerization to produce dark or brown products. Both growing conditions and genotype affect the expression of PPO activity (Lamkin *et al.* 1981, Baik *et al.* 1994, Park *et al*. 1997).

SUMMARY

Different stages of phenol colour reaction showed induction of phenol colour reaction occur post anthesis, i.e. stage 2. In the later stages, i.e. stage 3 (dough) and 4 (harvest maturity) seeds showed similar phenol reaction with less intensity. Phenol content estimated at different stages of growth and maturity showed the highest at post fertilization stage, i.e. stage 2 and it decreased in stage third and fourth. Inter-panicle phenol content was not significantly different whereas intra-panicle difference in phenol content was observed.

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REFERENCES

Abrol Y P and Uprety D C. 1970. Studies on darkening of whole wheat meal dough. *Current Science* **39**: 421–22.

Baik B K, Czuchajowska Z and Pomeranz Y. 1994. Comparison of polyphenol oxidase activities in wheats and flours from Australian and US cultivars. *Journal of Cereal Science* **19**(3): 291–96.

Bhowal J G, Banerjee S K and Joshi M G. 1969. The evolution of the phenol color reaction gene in wheat. *Japanese Journal of Genetics* **44**(3): 123–28.

Conn E E. 1964. *Enzymology of phenolic biosynthesis*. *Biochemistry of Phenolic Compounds* , pp 399–435. Academic Press, New York.

Harborne J B. 1964. *Biochemistry of Phenolic Compounds*. Academic Press, London-New York.

Joshi M G and Banerjee S K. 1970. Genetics of phenol colour reaction in emmer wheats. (In) *Proceedings of the International Seed Testing Association* **35**: 807-11.

Kruger J E. 1976. Changes in the polyphenol oxidases of wheat during kernel growth and maturation. *Cereal Chem* **53**: 201–13.

Kumar S J, Kumar G V, Dash A, Scholz P and Banerjee R. 2017. Sustainable green solvents and techniques for lipid extraction from microalgae: A review. *Algal Research* **21**: 138–47.

Lamkin W M, Miller B S, Nelson S W, Taylor D D and Lee M S. 1981. Polyphenol oxidase activities of hard red winter, soft red winter, hard red spring, white common, club, and durum wheat cultivars. *Cereal Chemistry* **58**: 27–31.

Oka H I. 1958. Intervarietal variation and classification of cultivated rice. *Indian Journal Genetics Plant Breeding* **18**: 79–89.

Park W J, Shelton D R, Peterson C J, Martin T J, Kachman S D and Wehling R L. 1997. Variation in polyphenol oxidase activity and quality characteristics among hard white wheat and hard red winter wheat samples. *Cereal Chemistry* **74**(1): 7–11.

Sivasubramanian S and Ramakrishnan V. 1974. Use of Phenol colour reactions for identification of rice varieties. *Experimental Agriculture* **10**(4): 299–303.

Singleton V L and Rossi J A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture* **16**(3): 144–58.

Steffens J C, Harel E and Hunt M D. 1994. Genetic engineering of plant secondary metabolism. *Polyphenol Oxidase,* pp.275–312. Ellis B E, Kuroki G W, Stafford H A, Boston M A (Eds), Springer, US.

Taneja S R, Abrol Y P and Sachar R C. 1974. Modulation of o-diphenolase and monophenolase enzymes during wheat grain development. *Cereal Chemistry* **51**: 457–65.

Vanangamudi K, Palanisamy V and Natesan P. 1988. Variety determination in rice—phenol and potassium hydroxide tests. *Seed science and Technology* **16**(2): 465–70.

Vaughn K C, Lax A R and Duke S O. 1988. Polyphenol oxidase: The chloroplast oxidase with no established function. *Physiol. Plant.* **72**: 659–65.

Walls W E. 1965. A standardized phenol method for testing wheat seed for variety purity. Handbook on Seed Testing. Association of Official Seed Analysts.