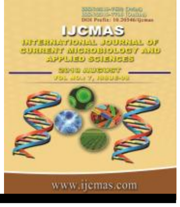
Int.J.Curr.Microbiol.App.Sci (2018) 7(8): 1172-1186

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 7 Number 08 (2018)**
Journal homepage: http://www.ijcmas.com



Original Research Article

https://doi.org/10.20546/ijcmas.2018.708.132

Identification of Transgressive Segregants with High Zinc in Grains under Aerobic Condition in F_4 Population of Rice (*Oryza sativa* L.)

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ABSTRACT

Keywords

Biofortification, GCV, PCV, zinc, rice (*Oryza sativa* L.)

Article Info

Accepted:
08 July 2018
Available Online:
10 August 2018

Progenies from a cross between '*Gopaldoddiga*' and 'ARB6' cultivars were selected on the basis of combining ability analysis to study the genetics of transgressive segregation for zinc content and agronomic traits using augmented designs. Results revealed the presence of general combining ability and specific combining ability effects, and the parents were found to be good general combiners for zinc in grains, 100 grains weight and grain yield per plant. Higher phenotypic and genetic coefficients of variation for all characters, and high heritability coupled with high genetic advance for most of the traits indicates strong additive genetic control of these traits. Presence of positive relationship between grain yield and grain Zn concentration implies these traits can be used as a selection criterion from F_4 generation onwards. These zinc enriched high yielding hybrids can be effectively utilized in the rice biofortification programs.

Introduction

Rice is the most staple food for nearly half of the world's population (Bi and Yang, 2017) standing the third-highest worldwide production, after sugarcane and maize (FAOSTAT, 2012). Rice is presently grown on 144 million hectares throughout the continent, with China and India dominating with over half of the total area harvested (FAO, 2016). Nearly 90% of the total rice consumption is in Asian countries, where it is a staple food for a majority of the population.Rice, therefore, is of special importance for the nutrition of large reaches of the population (FAO, 2006). However, rice is a poor source of vitamins and essential micronutrients such as Zn (Muthayya *et al.*, 2014).

Micronutrient deficiencies or hidden hunger has become a major nutritional problem affecting more than two billion people in the developing countries of Asia, Africa, and Latin America (Swamy *et al.*, 2016). Zinc (Zn) is one of the essential micronutrients, which serves as a co-factor for more than 300 enzymes involved in the metabolism of carbohydrates, lipids, proteins, and nucleic acids, hence its importance in normal growth and development of plants and animals (Roohani *et al.*, 2013; Sadeghzadeh, 2013).

One-third of the population, particularly children and women suffer from Zn deficiency related health problems such as growth retardation, loss of appetite, impaired immune function, hair loss, diarrhea, eye and skin lesions, weight loss, delayed healing of wounds, and mental lethargy (Maret and Sandstead, 2006; Prasad, 2003; Wang and Bushey, 2005).

Most of the rice growing areas are Zn deficit and Zn availability in irrigated rice ecosystems is very low due to formation of less soluble Zn complexes under anaerobic conditions. Various approaches have been developed in recent years with the aim of high Zn biofortified rice genotypes to accumulate Zn in grains. Agronomic Zn biofortification through Zn fertilizer application is a complementary approach to increase grain Zn concentration in new elite rice genotypes to ensure adequate root Zn uptake and transport to the grains during reproductive growth stage (Phattarakul *et al.*, 2012; Shivay *et al.*, 2008), but there are higher expenditure associated with the application. In addition, zinc fertilizer application effect can be impaired by physical and chemical characteristics of soil, which reduce the availability of Zn to plants, leading to a disappointing experience for farmers (Henriques *et al.*, 2012). Genetic engineering techniques may be used to biofortify the crops with minerals like iron and Zn (Tiwari *et al.*, 2010) as transgenic rice lines showed an improvement of 15–30 mg kg^{-1} in Zn levels(Johnson *et al.*, 2011; Masuda *et al.*, 2012 and Slamet-Loedin *et al.*, 2015). At the moment, the application of genetic transformation (or genetic engineering) is seriously hindered because there is controversy on food safety and environmental impacts over any genetically modified (GM) crop (Nicolia *et al.*, 2014).

The genetic biofortification strategy uses plant breeding techniques to produce staple food crops with higher micronutrient levels (Harvest Plus, 2014). The world’s first Zn enriched rice variety was released in 2013 by the Bangladesh Rice Research Institute (BRRI dhan 62), which is claimed to contain 20–22 mg Zn kg^{-1} for brown rice. Nonetheless this is short of the target of 30 mg Zn kg^{-1} set by the Harvest Plus program (Shahzad *et al.*, 2014). With the aim of further enrichment of Zn in commercial cultivars, in this study we estimate Zn content in grains, analyze correlation between Zn and grain yield, selection of genotypes for high Zn productivity of F_4 segregating populations.

Materials and Methods

The experiment was carried out during *Kharif* season of 2016using augmented experimental design as described by Federer (1961) under aerobic condition at the field of the Department of Plant Biotechnology University of Agricultural Sciences, GKVK, Bengaluru. Ninety seven transgressive segregants of F_4 population derived from Gopaldoddiga x ARB6cross were sown in 10 blocks using Gopaldoddiga, ARB6, AM 143 and AM65 as checks under aerobic condition.

Observations were recorded on following attributes *viz*: days to 50 *per cent*flowering, days to maturity, plant height (cm), number of tillers per plant, number of productive tillers per plant, panicle length (cm), grain yield per plant (g) at appropriate stages of crop. Biomass of plant (g), harvest index (%), 100 grains weight (g) and brown rice Zn mg kg^{-1}were recorded after harvest.

Grains of individual lines were harvested manually and hand threshed to avoid any contamination. The grains were then manually dehusked. Unbroken, uniform grains were then washed in dilute hydrochloric acid followed by washing with double distilled water to remove any surface contaminants and dried in hot air oven at 70 °C for 72 hours. The Zn content in these grains was estimated using X-ray florescence (XRF) (Paltridge *et al.*, 2012). Five grams of brown rice from each plant was subjected to the XRF and content (mg kg^{-1}) was recorded. The experimental data was compiled by taking mean values of three replications for each genotype.

The analysis of variance for different characters was computed as suggested byRana *et al.*, (1991). Both phenotypic and genotypic coefficient of variability for traits was estimated using the formulae of Burton and DeVane (1953). Heritability and genetic advance were calculated as per the method outlined by Hanson *et al.*, (1956).Phenotypic coefficients of correlation between various characters were obtained as suggested by Al-Jibouri *et al.*, (1958). Path coefficient analysis was carried out following the method of Dewey and Lu (1959).

Results and Discussion

Analysis of Variance (ANOVA)

Results from analysis of variance for growth traits, grain zinc content and yield attributing traits inF_4generation of Gopaldoddiga X ARB6are presented in Table 1. Mean sum of squares of progenies exhibited highly significant difference for all the traits in the cross under study. Analysis of variance for both progenies and checks, and checks *versus* progenies also displayed significant difference for characters such asday to 50% flowering, plant height, number of productive tillers, panicle length, grain yield per plant, harvest index, 100 grains weight, biomass per plant and brown rice zinc. This indicates that the differences occur between genotypes, not simply because of environmental influences. The performance of checks or F_4 generation will be influenced by two factors: the genetic properties it carries and the environment where it is cultivated; if the environment is uniform, the plant character will be influenced only by the genetic properties. Similar findings were reported earlier by Bekele *et al.*, (2013), Rashid *et al.*, (2017) and Barokah *et al.*, (2018).

Genetic variability parameters

Higher estimates (>15%) of GCV and PCV were observed for biomass per plant, grain yield per plant, total number of tillers, number of productive tillers and harvest index (Figures 1 and Table 2). GCV and PCV estimates were moderate (<15%) for 100 grains weight, plant height, day to 50% flowering, panicle length and brown rice zinc. The PCV values were only slightly higher than GCV values, so the similar magnitude of PCV and GCV for all traits suggested that these characters were under the strong influence of genetic control and less influence of the environment. Thus individual plant selection can be practiced for these characters. Similar results were reported by Bisne *et al.*, (2009), Akinwale *et al.*, (2011), Govindharaj *et al.*, (2016), Revathi *et al.*, (2016), Yugandhar *et al.*, (2017), Prasad *et al.*, (2017), Abebe *et al.*, (2017), Rajpoot *et al.*, (2017), Ajmera *et al.*, (2017), Nandeshwar *et al.*, (2010), Shet *et al.*, (2012), Kiran *et al.*, (2013), Bekele *et al.*, (2013), Tuhina-Khatun *et al.*, (2015) and Mamata *et al.*, (2018).

High PCV and GCV was observed for grain zinc content, which is consistent with previous reportsPurusothaman *et al.*, (2010); Samak *et al.*, (2011); Shashidhara *et al.*, (2013), Sala *et al.*, (2014), Anjali (2017), Ajmera *et al.*, (2017); Madhubabu *et al.*, (2017) and Shashidhara *et al.*, (2017).

Heritability and genetic advance

The estimates of heritability act as predictive instrument in expressing the reliability of phenotypic selection. Therefore, high heritability helps in effective selection for a particular character. In this study, high estimates of broad sense heritability along with high genetic advance (expressed as *per cent* of mean) was observed for day to 50% flowering, plant height, total number of tillers, number of productive tillers, panicle length, grain yield per plant, harvest index, biomass per plant and brown rice zinc (Figure 2 and Table 2). It indicates the presence of strong additive gene effects and there is potential for genetic improvement of these traits in future breeding programmes. From the results of the present study, it can be concluded that single plant selection could be effectively made as environment does not have any significant influence in the variation of traits. High heritability and genetic advance as per cent of mean was earlier reported by Babu *et al.,* (2012), Bekele *et al.,* (2013), Sadimantara *et al.,* (2014); Soman *et al.,* (2015); Limbani *et al.,* (2017); Yadav *et al.,* (2017); Sumanth *et al.,* (2017) and Shamim *et al.,* (2017).

Correlation of Zinc plant with growth parameters and yield component characters

Highly significant and positive phenotypic correlations were observed for biomass per plant, harvest index with grain yield (Table 3). These traits may indirectly contribute for increased grain yield. These were in accordance with the results of Bekele (2012) and Ashlesha (2015).

Brown rice zinc showed a positive correlation with day to 50% flowering, total number of tillers, number of productive tillers, biomass, grain yield per plant, harvest index and 100 grains weight were consistent with the reports of Tiwari *et al.,* (2010) and Morete *et al.,* (2011). Thus, it can be concluded that it is possible to develop high yielding varieties with high levels of Zn.

Gregorio (2002) reported that a positive relationship between grain yield and grain Zn concentration was observed under Zn-deficient soil. From the results of the present study, assessment of the relationship between brown rice zinc and grain yield per plant using linear regression showed that there was a positive correlation between these traits (Figure 3). Hence, these characters could be considered as criteria for selection for higher yield as they were mostly interrelated positively in addition to a positive association with grain zinc. This result was in conformity with the results of Rathod *et al.,* (2017) and Ajmera *et al.,* (2017).

Figure.1 Graphical representation of phenotypic (PCV) and genetic (GCV) coefficients of variation

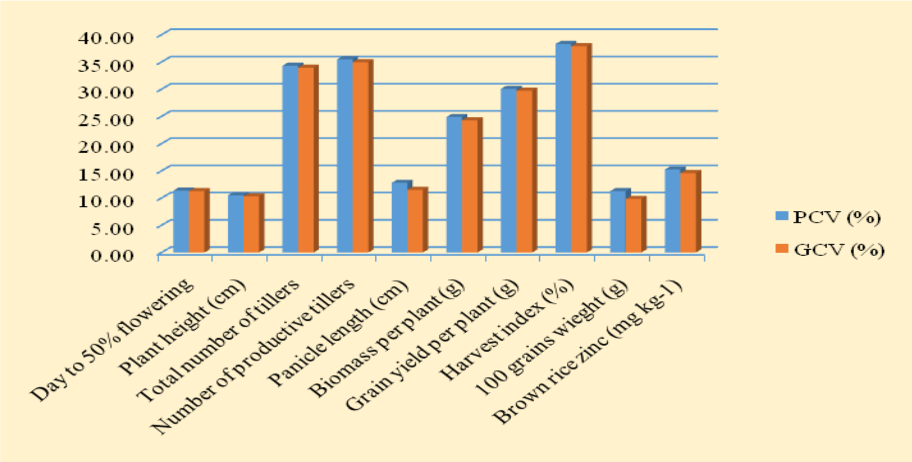

Figure.2 Graphical representation of heritability and genetic advancement as percentage of mean (GAM)

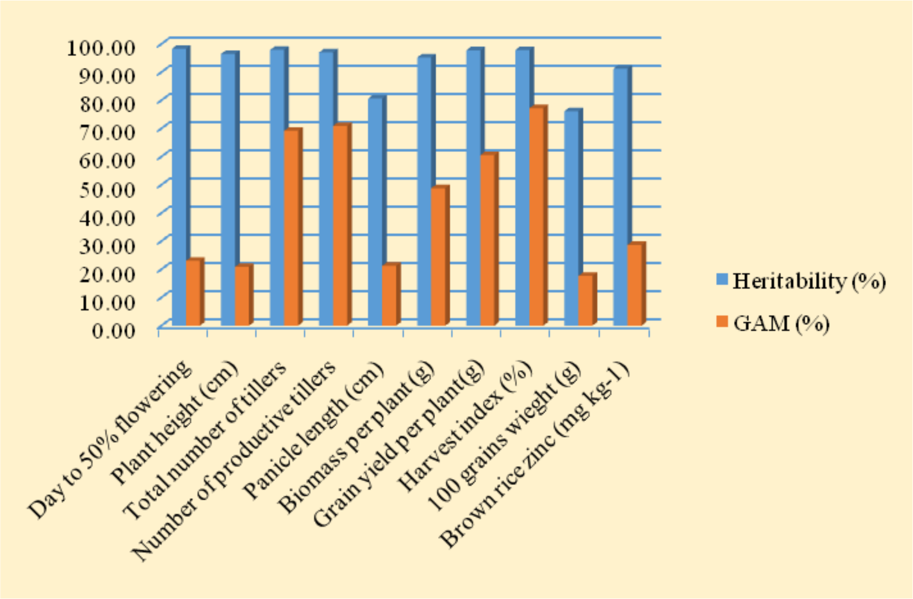

Figure.3 Relationship between brown rice zinc and grain yield per plant in F_4 population of Gopaldoddiga X ARB6 in Kharif-2016

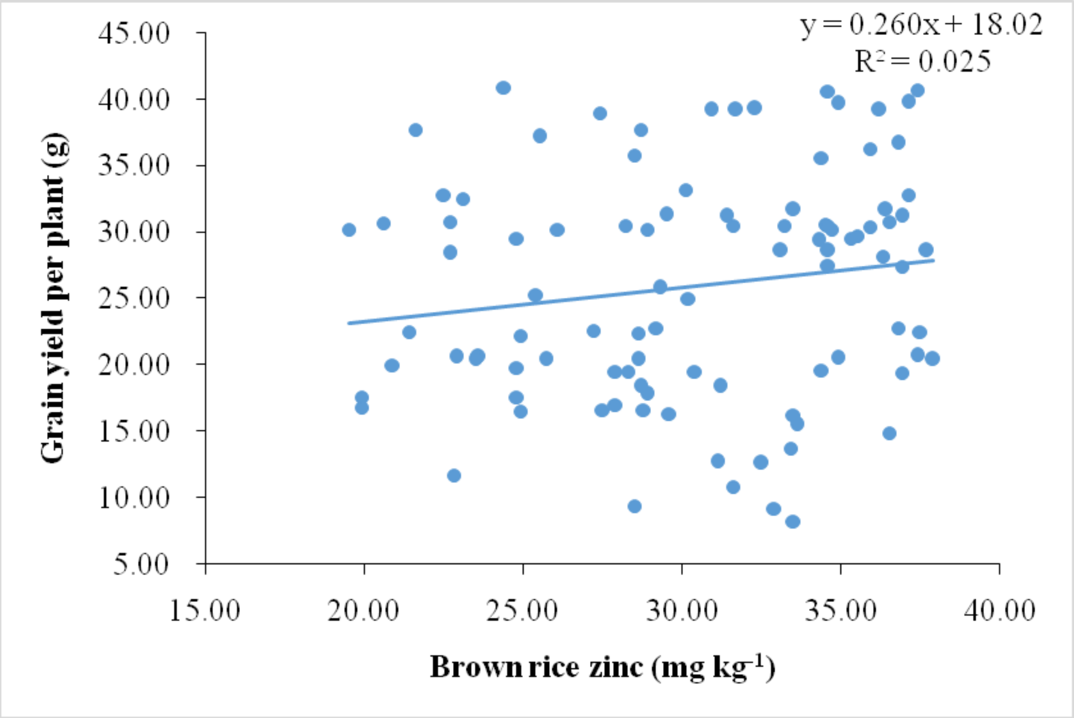

Table.1 Analysis of variance (mean sum of squares) for 10 different characters in F_4 population of Gopaldoddiga X ARB6 in *Kharif*-2016

Source of variation	Df	Mean sum of square									
		Day to 50% flowering		**Plant height**		**Total number of tillers**		**Number of productive tillers**		**Panicle length**	
Blocks (eliminating check + var)	9	3.111		4.871		1.456		1.469		1.051	
Progenies + Checks	103	199.134	***	337.325	***	122.456	***	105.045	***	20.335	***
Checks	3	2142.892	***	7272.637	***	445.433	***	417.558	***	332.761	***
Progenies	99	131.768	***	121.682	***	96.121	***	85.163	***	9.511	***
Checks vs. Progenies	1	1037.161	***	880.071	***	1760.643	***	1135.801	***	154.626	***
Error	27	1.708		3.287		1.544		1.929		1.469	
Source of variation	**Df**	**Mean sum of square**									
		Biomass per plant		**Grain yield per plant**		**Harvest index**		**100 grains wieght**		**Brown rice zinc**	
Blocks (eliminating check + var)	9	4.335		0.955		0.001		0.025		2.544	
Progenies + Checks	103	160.683	***	120.501	***	0.136	***	0.179	***	39.402	***
Checks	3	1964.702	***	352.343	***	0.068	***	1.110	***	130.268	***
Progenies	99	107.000	***	76.769	***	0.102	***	0.109	***	27.283	***
Checks vs. Progenies	1	63.240	***	3754.460	***	3.751	***	4.277	***	966.619	***
Error	27	3.928		1.317		0.002		0.021		1.854	

* Significant at 5%; ** Significant at 1%; *** Significant at 0.1%
Df: Degrees of freedom.

Table.2 Estimate of genetic parameters for different traits inF_4 population of Gopaldoddiga X ARB6 in Kharif-2016

Sl.No.	Plant characters	Min.	Max.	Mean ± S.E			GCV (%)	PCV (%)	h2 (%)	GAM (%)
1	Day to 50% flowering	70.00	108.00	88.21	±	1.16	11.27	11.37	98.31	23.02
2	Plant height (cm)	73.80	120.40	92.26	±	1.12	10.31	10.50	96.48	20.87
3	Total number of tillers	7.00	40.00	25.28	±	0.99	33.95	34.31	97.90	69.19
4	Number of productive tillers	7.00	39.00	23.02	±	0.94	34.95	35.48	97.05	70.93
5	Panicle length (cm)	16.67	28.67	21.52	±	0.32	11.50	12.80	80.65	21.27
6	Biomass per plant (g)	16.60	59.30	36.66	±	1.06	24.27	24.87	95.23	48.79
7	Grain yield per plant(g)	3.70	40.60	21.32	±	0.93	29.72	30.06	97.76	60.53
8	Harvest index (%)	0.19	0.78	0.57	±	0.01	37.88	38.29	97.86	77.20
9	100 grains weight (g)	1.80	3.60	2.64	±	0.03	9.83	11.27	76.13	17.67
10	Brown rice zinc (mg kg^{-1})	19.50	37.90	30.33	±	0.53	14.57	15.25	91.26	28.68

* Significant at 5%; PCV = Phenotypic Coefficient of variation; GCV= Genotypic Coefficient of variation; h^2 % = Heritability percentage in broad sense; GAM: Genetic Advance as per Mean.

Table.3 Estimates of phenotypic correlation coefficients for different quantitative traits in F_4 population of Gopaldoddiga X ARB6 in *Kharif*-2016

	Plant height (cm)	Total number of tillers	Number of productive tillers	Panicle length (cm)	Biomass per plant (g)	Grain yield per plant (g)	Harvest index	100 grains weight (g)	Brown rice zinc (mg kg^{-1})
Day to 50% flowering	-0.931**	0.977**	0.974**	0.225*	0.05	0.10	0.10	-0.851**	0.11
Plant height (cm)	1	-0.943**	-0.949**	-0.15	-0.09	-0.14	-0.11	0.804**	-0.10
total number of tillers		1	0.994**	0.288**	0.05	0.10	0.09	-0.846**	0.11
Number of productive tillers			1	0.254*	0.06	0.11	0.10	-0.854**	0.10
Panicle length (cm)				1	-0.03	-0.01	0.03	-0.223*	-0.05
Biomass per plant (g)					1	0.77**	0.20	-0.03	0.20
Grain yield per plant(g)						1	0.76**	-0.08	0.19
Harvest index							1	-0.08	0.09
100 grains weight (g)								1	0.13

* Significant at 5%; ** Significant at 1%

Table.4 Estimates of phenotypic path coefficient analysis for different quantitative traits in F_4 population of Gopaldoddiga X ARB6 in *Kharif*-2016

	Day to 50% flowering	Plant height (cm)	Total number of tillers	Number of productive tillers	Panicle length (cm)	Biomass per plant (g)	Harvest index	100 grains weight (g)	Brown rice zinc (mg kg^{-1})
Day to 50% flowering	**-0.042**	0.039	-0.041	-0.041	-0.010	-0.002	-0.004	0.036	-0.005
Plant height (cm)	0.020	**-0.021**	0.020	0.020	0.003	0.002	0.002	-0.017	0.002
Total number of tillers	0.131	-0.126	**0.134**	0.133	0.039	0.006	0.013	-0.113	0.015
Number of productive tillers	-0.109	0.106	-0.111	**-0.112**	-0.028	-0.007	-0.011	0.095	-0.011
Panicle length (cm)	-0.005	0.003	-0.006	-0.006	**-0.022**	0.001	-0.001	0.005	0.001
Biomass per plant (g)	0.032	-0.058	0.030	0.038	-0.017	**0.646**	0.128	-0.018	0.126
Harvest index	0.060	-0.068	0.060	0.062	0.017	0.126	**0.633**	-0.050	0.055
100 grains weight (g)	0.012	-0.011	0.012	0.012	0.003	0.000	0.001	**-0.014**	-0.002
Brown rice zinc (mg kg-1)	0.001	-0.001	0.001	0.001	0.000	0.001	0.001	0.001	**0.007**

Table.5 Transgressive lines selected from F_4 population of Gopaldoddiga X ARB6 based on grain zinc content grown in Kharif-2016

SL. No.	Superior plants	Day to 50% flowering	Plant height (cm)	Total number of tillers	Number of productive tillers	Panicle length (cm)	Biomass per plant (g)	Grain yield per plant (g)	Harvest index	100 grains weight (g)	Brown rice zinc (mg kg^{-1})
1	GA 247-12-101	99.00	100.00	18.00	16.00	19.00	29.90	8.60	0.29	2.83	37.70
2	GA 247-12-141	80.00	97.00	20.00	18.00	19.67	23.40	10.70	0.46	2.80	37.40
3	GA 247-12-984	77.00	100.40	11.00	10.00	17.67	28.40	19.40	0.68	3.04	36.90
4	GA 240-450-35	93.00	81.80	34.00	31.00	20.33	40.20	20.40	0.51	2.37	37.90
5	GA 240-450-58	100.00	87.20	31.00	29.00	24.00	25.50	12.80	0.50	2.45	36.80
6	GA 240-450-21	104.00	89.40	39.00	37.00	25.33	30.40	22.50	0.74	2.77	37.50
7	GA 287-621-48	75.00	109.40	12.00	12.00	18.00	47.00	14.70	0.31	2.93	37.40
8	GA 287-63-172	96.00	80.80	35.00	31.00	28.67	33.00	16.70	0.51	2.34	36.40
9	GA 287-63-245	108.00	82.60	38.00	35.00	24.33	54.60	36.70	0.67	2.16	36.80
10	GA 287-63-25	87.00	91.80	24.00	22.00	21.33	32.80	10.80	0.33	2.65	36.50
11	GA 214-132-20	99.00	82.80	32.00	30.00	28.67	33.90	12.80	0.38	2.42	37.10
12	GA 214-132-51	108.00	81.40	38.00	35.00	24.33	34.60	13.20	0.38	2.11	36.90
13	GA 214-132-136	99.00	76.20	36.00	33.00	24.00	50.00	29.80	0.60	2.26	37.10
14	GA 214-132-26	101.00	84.40	36.00	32.00	23.33	30.00	14.90	0.50	2.34	36.50
15	GA 214-132-279	75.00	110.40	11.00	9.00	17.00	46.80	27.40	0.59	3.15	36.90
Check -1	*Gopaldoddiga*	72.5	100.68	8.10	7.80	24.11	26.54	6.80	0.26	2.72	29.74
Check - 2	ARB 6	99.4	80.04	21.1	19.9	18.72	27.57	12.45	0.45	1.96	22.27
Parent - 1	AM 143	102.2	75.22	23.1	22.6	20.75	41.48	16.05	0.39	2.08	22.19
Parent - 2	AM 65	103.2	134.52	16.3	15.6	31.84	56.36	20.88	0.37	2.22	23.33

Path coefficient analysis

Using path coefficient analysis, correlation between two variables can be partitioned into their direct and indirect effects through other traits (Wright, 1921). In this study we calculated direct and indirect effects of yield and yield contributing characters. When the magnitude of relationship between a casual factor and the effect is almost equal to its direct effect, it explains the true relationship and a direct selection through this trait can be applied. However, when the correlation is positive, but the direct effect is negative or negligible, the indirect effects apparently cause that positive correlation. In such situation the other factors are to be considered simultaneously for selection. When the correlation coefficient is negative but direct effect is positive and high, we need to apply some restriction to nullify the undesirable indirect effects in order to make use of direct effect.

The phenotypic path-coefficient analysis indicated high positive direct effect of biomass per plant (0.646), harvest index (0.633) and total number of tillers (0.134) on grain yield per plant (Table 4). These results are in agreement with Solomon and Wegary (2016), Muthuramu and Sakthivel, (2016) and Soman *et al.*, (2014). Number of productive tillers (-0.112), 100 grains weight (-0.014) had negative direct effect of on grain yield per plant. Similar result was reported by Muthuvijayaragavan and Murugan (2017). Path-coefficient analysis gives information for the direct and indirect effects of different traits on grain yield. The trait brown rice zinc (0.007) expressed direct effects on grain yield. This was in conformity with the findings of Ashlesha (2015) and Rathod *et al.*, (2017). This indicated that grain zinc concentration does not have any role in enhancing grain yield per plant.

Selected superior segregants in F_4segregating generations

Ten progenies with high zinc in grain as well as some additional important traits such as day to 50% flowering, plant height, total number of tillers, number of productive tillers, panicle length, biomass per plant, grain yield per plant, harvest index, 100 grains weight and brown rice zinc, were selected from F_4 segregating populations (Table 5). From the selection it was observed that high yielding progenies have higher brown rice zinc per plant. As these progenies were still segregating, more generations need to be tested before releasing for multi-location trial.

Acknowledgements

We sincerely thank the Department of Biotechnology, Ministry of Science & Technology, New Delhi, India for providing the financial support.

Conflict of interest

The authors declare that they have no conflict of interest.

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How to cite this article:

Thuy, N.P., D.B. Santhosh and Shashidhar, H.E. 2018. Identification of Transgressive Segregants with High Zinc in Grains under Aerobic Condition in F_4 Population of Rice (*Oryza sativa* L.). *Int.J.Curr.Microbiol.App.Sci.* 7(08): 1172-1186.
doi: https://doi.org/10.20546/ijcmas.2018.708.132