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Rice Grain Chalkiness Is Negatively Correlated with Root Activity During Grain Filling

ZHONG Xu-hua, HUANG Nong-rong
(*Rice Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China*)

Abstract: Field and pot experiments were conducted using indica rice varieties with differential chalkiness. There were significant differences in root activity among the varieties. The percentages of chalky grains and chalky area were both negatively correlated with root activity expressed as α-naphthylamine oxidation ability (RA) per gram of fresh root (RA_{fw}) , RA per spikelet (RA_{grn}), or RA per sink capacity (RA_{sink}). The RA_{sink} was more closely related to chalkiness than RA_{fw} and RA_{grn} when varieties differed greatly in panicle size and grain weight. Application of NO_3^--N fertilizer at heading resulted in higher root activity and reduced chalkiness. Application of 30 mg/L NaN_3 (respiration inhibitor) resulted in reduced root activity and increased chalkiness for one variety 'GD9501', but for the other variety 'Qinluai' was in reverse. The percentages of chalky grains and chalky area were negatively correlated with root activity at 10 days after heading under different chemical treatments (r =－0.8567* and r =－0.9211**, respectively).
Key words: chalkiness; root activity; nitrate nitrogen; sodium azide; rice (*Oryza sativa*)

Good grain quality is important for farmer's profitability of rice production, especially for early season rice in South China [1]. Chalkiness affects the appearance quality of milled rice and is a main character determining grain price. Chalky grains tend to be broken easily during processing, which results in low head rice rate [2, 3]. Studies on the mechanisms of chalkiness formation have mainly been focused on source-sink balance and grain filling [4-9]. It is revealed that chalkiness is mainly determined by the activity of source (leaves) and sink (grain) and the grain filling dynamics [4, 8, 9], which are closely related to root activity during grain filling [10-14]. A relationship between chalkiness and root activity has been expected. The purpose of this research is to test the hypothesis that chalkiness is correlated with root activity during grain filling.

MATERIALS AND METHODS

Field experiment

A field experiment was conducted during late season (from July to November) in 2000 at Dafeng Experimental Farm of Guangdong Academy of Agricultural Sciences, Guangzhou, China (113°18′ E, 23°10′ N, elevation 18 m). Four indica varieties were arranged in a randomized complete block design with three replicates. Among the four varieties, Aojingzhan and Wufengzhan have low chalkiness, and IR66159-189-5-5-3 and Tesan'ai 2 have high chalkiness. Seeds were sown on 24 July. Transplanting was done on 12 August at a spacing of 20 cm×13.3 cm with four seedlings per hill. Plot size was 8 m^2.

Measurement of root amount and activity

Number of effective stems was counted at full heading. Five hills with average stem number were taken from each plot at full heading, milky ripening, and maturity[12]. Roots were carefully taken from the field and washed. Root fresh weight (FW_{rt}, g / m^2) was recorded and α-naphthylamine (α-NA) oxidation ability (RA_{fw}, μg / g·h) was measured. Total root activity (TRA, μg / m^2·h)was calculated as follows: $TRA = FW_{rt} \times RA_{fw}$.

Measurement of spikelet number and sink capacity

Number of panicles (NP, panicles/m^2) was counted at maturity. Five hills were sampled and number of grains (including both filled and unfilled)

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Corresponding author: ZHONG Xu-hua (xzhong1@pub.guangzhou.gd.cn)

per panicle (N_{grn}) and grain weight (GW, g/grain) were measured.

Total grain number (TGN, No. / m^2) = NP × N_{grn},

Sink capacity (SS, g / m^2) = TGN × GW,

Root activity per grain (RA_{grn}, μg / grain · h) =TRA / TGN,

Root activity per gram of sink (RA_{sink}, μg / g·h) = TRA / SS.

Measurement of chalkiness

For each plot, 30 hills were harvested and grains were dried. The chalky grain rate and chalky area were measured following the standard procedure [15].

Pot experiment

The experiment was conducted in the net house of the Rice Research Institute of Guangdong Academy of Agricultural Sciences in Guangzhou during early season (March–July) in 2001. The results of the experiments in 2000 showed that sink capacity (panicle size and grain weight) significantly affected chalkiness. To avoid this effect, two varieties with different chalkiness but with similar panicle size and grain weight were used. GD9501 was a variety with large chalkiness while Qingliu'ai with small chalkiness. Seeds were sown on 5 March and seedlings were transplanted on 4 April to plastic pots filled with 15 kg soil. The pot was 30 cm in diameter and 30 cm in height. There were three treatments for each variety. Each treatment had eight pots. In each pot five hills were planted with two seedlings per hill. Treatments were: T1, no treatment (CK); T2, 0.6 g fertilizer-N applied for each pot in the form of $Ca(NO_3)_2 \cdot 4H_2O$ at heading; T3, 250 mL NaN_3 (30 mg/L, respiration inhibitor) applied for each pot at heading. Chemicals were carefully applied so that leaves were not contaminated. Five hills were sampled and root activity was measured at 10 and 20 d after heading and at maturity. At maturity grains were harvested and dried and chalkiness was measured.

RESULTS

Differences in chalkiness among varieties

There were significant differences in chalkiness among the four varieties (Table 1). Tesan'ai 2 had the highest chalkiness, while Aojingzhan had the lowest. The percentage of chalky grains and chalky area of Tesan'ai 2 were 10 times greater than those of Aojingzhan.

Differences in root activity among varieties

In this paper, root activity (RA) is expressed as α-naphthylamine oxidation ability per gram of fresh root (RA_{fw}), per grain (RA_{grn}) or per sink capacity (RA_{sink}) (Table 2). Wufengzhan 2 had the greatest RA_{fw} at heading, significantly greater than that of

Table 1. Percentages of chalky grains and chalky area.

Variety	Chalky grains (%)	Chalky area (%)
Aojingzhan	9.33 c	3.03 c
Wufengzhan 2	12.33 c	4.54 c
IR66159-189-5-5-3	44.33 b	7.87 b
Tesan'ai 2	99.00 a	32.68 a

Within a column, values followed by the same letter are not significantly different at 0.05 probability level.

Table 2. Changes in root activity of varieties with different chalkiness during grain-filling stage.

Variety	Root activity per gram of fresh root (μg / g • h)			Root activity per grain (μg / grain • h)			Root activity per sink capacity (μg / g • h)		
	Heading	Milky ripening	Maturity	Heading	Milky ripening	Maturity	Heading	Milky ripening	Maturity
Aojingzhan	45.17 b	28.62 a	40.23 a	0.40 b	0.32 b	0.21 a	22.51 bc	18.33 b	11.82 b
Wufengzhan 2	72.07 a	52.25 a	41.72 a	0.70 a	0.71 a	0.41 a	38.72 a	39.30 a	22.65 a
IR66159-189-5-5-3	56.66 ab	30.69 a	39.68 a	0.62 ab	0.33 b	0.31 a	26.90 b	14.32 b	13.58 b
Tesan'ai 2	41.62 b	36.29 a	54.00 a	0.42 b	0.39 b	0.37 a	14.55 c	13.60 b	12.60 b
$LSD_{0.05}$	17.865	28.453	19.208	0.235	0.230	0.171	11.276	11.272	7.647

Within a column, values followed by the same letter are not significantly different at 0.05 probability level.

Table 3. Correlation coefficients between chalkiness and root activity during grain-filling (*n*=12).

Chalkiness	Root activity per gram of fresh root			Root activity per grain			Root activity per sink capacity		
	Heading	Milky ripening	Maturity	Heading	Milky ripening	Maturity	Heading	Milky ripening	Maturity
Chalky grain rate	-0.4514	-0.1399	0.5656	-0.2319	-0.2679	0.3090	-0.5843*	-0.5315	-0.2610
Chalky area	-0.4549	-0.0680	0.6053*	-0.2985	-0.1766	0.2973	-0.5882*	-0.4264	-0.2380

* Significant at 0.05 probability level.

Table 4. Percentage of chalky grains, chalky area and root activity during grain-filling of rice treated with different chemicals.

Variety	Treatment	Percentage of chalky grains (%)	Percentage of chalky area (%)	Root activity (μg / g·h)		
				10 d after heading	20 d after heading	Maturity
GD9501	CK	79.5	28.7	161.6 (35.8)	128.4 (5.8)	78.4 (8.0)
	NO_3^--N	83.0	28.2	174.6 (13.4)	133.0 (31.6)	119.0 (27.2)
	NaN_3	95.0	36.6	135.0 (23.6)	116.5 (8.3)	55.6 (11.7)
Qingliu'ai	CK	40.5	18.7	175.1 (33.1)	140.6 (10.2)	48.4 (5.6)
	NO_3^--N	33.5	16.2	217.1 (29.0)	173.8 (23.8)	96.6 (34.5)
	NaN_3	40.0	15.4	216.1 (34.5)	126.5 (32.9)	100.1 (21.0)

Values in the parentheses are standard errors.

Aojingzhan and Tesan'ai 2. The RA_{fw} at milky ripening and maturity was not significantly different among varieties. Wufengzhan 2 had the greatest RA_{grn} at all the three sampling dates. The differences were significant among varieties at 10 and 20 days after heading. At maturity there were no significant differences in RA_{grn} among the four varieties. However, the differences in RA_{sink} were significant among the four varieties at all the three sampling dates. Wufengzhan 2 had the highest RA_{sink} at all the sampling dates.

Relationship between chalkiness and root activity during grain filling

The RA_{fw}, RA_{grn}, and RA_{sink} at heading and milky ripening were negatively correlated with the percentages of chalky grains and chalky area (Table 3). Chalkiness decreased as root activity increased. The correlation coefficients varied greatly when different root activity indices were used. At full heading and milky ripening, the correlation coefficients with RA_{sink} were consistently greater than those with RA_{fw} or RA_{grn}. At full heading the correlation of RA_{sink} with the percentage of chalky grains and with the chalky area were both significantly negative ($P<0.05$).

Effect of chemical treatments on chalkiness and its relation to root activity

In the pot experiment, GD9501 had a higher chalkiness than Qingliu'ai in all treatments (Table 4). Application of NO_3^--N improved root activity for both varieties during grain filling, especially at maturity. NO_3^--N had little effect on the chalkiness of GD9501, while reduced the chalkiness of Qingliu'ai significantly. NaN_3 application resulted in reduced root activity and increased chalkiness for GD9501. For Qingliu'ai, NaN_3 decreased root activity only at 20 days after heading. Root activity of Qingliu'ai was unexpectedly increased at 10 days after heading and maturity and chalkiness of Qingliu'ai was reduced. The reason for this phenomenon is unknown. Chalkiness was negatively correlated with root activity for both GD9501 and Qingliu'ai under chemical treatments (Table 5).

Table 5. Correlation coefficients between chalkiness and root activity during grain-filling.

Chalkiness	Days after heading		
	10 d	20 d	30 d
Chalky grain rate	-0.8567*	-0.6761	-0.0678
Chalky area	-0.9211**	-0.6273	-0.2454

*,** Significant at 0.05 and 0.01 probability level, respectively.

DISCUSSION

This study showed that rice grain chalkiness was negatively correlated with root activity during grain filling. This was true both among varieties with different root activity and chalkiness and among chemical treatments resulting in different root activity and chalkiness. This result implies that root activity should be considered in good-quality rice breeding and crop cultivation. Breeders may take high root activity and slow senescence of root activity during grain filling as a selection criterion in crop improvement, and farmers can reduce chalkiness and improve grain quality by enhancing root activity during grain filling through NO_3^--N fertilizer application or favorable water management. Breeders and farmers have recognized that early root senescence usually results in higher chalkiness. The results of this study were consistent with such observations.

We used RA_{fw}, RA_{grn}, and RA_{sink} as indices of root activity to evaluate the relationship between chalkiness of different varieties and root activity during grain filling. The relationships between chalkiness and root activity at different sampling dates were not consistent when RA_{fw} or RA_{grn} were used. It became consistent when RA_{sink} was used (Table 3). This indicated that RA_{sink} is more suitable in representing root activity than RA_{fw} and RA_{grn}. The RA_{sink} is an integration of four factors, i.e. root activity per gram of fresh root, fresh weight of root, number of grains per m^2, and grain size expressed as grain weight. This explains why it represents root activity better. However, RA_{sink} is more laborious to measure compared with RA_{fw} and RA_{grn}.

It is well known that rice grain chalkiness can result either from insufficient source supply for grain filling or inappropriate grain filling dynamics [8, 9]. Grain filling is affected by root activity, which is positively related to photosynthetic rate [10] and crop growth rate [11]. Increased root activity slows down the decomposition of chlorophyll, maintains great leaf area index, and stimulates dry matter production for grain filling[13]. In addition, increased root activity enhances the translocation of carbohydrates from stems and sheaths to growing grains. Less ^{14}C-labelled carbohydrate is remained in leaves when root activity is high [12, 16]. Grain filling strength is positively correlated with root activity [12]. Filled grain percentage and 1000-grain weight are greater for the plants with high root activity [10, 11, 14]. Increasing RA_{sink} is an effective approach to improve grain filling and 1000-grain weight [11]. These might be the physiological basis for the negative correlation between chalkiness and root activity.

Root activity differed greatly between the two experimental years (cf. Table 2 and Table 4). This might have been caused by variety, season, and/or planting methods. There might be great differences in root activity among varieties. We measured root activity of five varieties at heading during early season in 1998. The highest root activity achieved by Yuexiangzhan was 125 μg/g·h, while the lowest by Pei'ai 64S / E32 was 76 μg/g·h, being 40% lower than Yuexiangzhan [17]. Root activity is also greatly affected by crop management. It was reported that root activity was much higher under the cultivation of system of rice intensification (SRI) than under conventional management [18]. In our experiments, plants grew better during 2001 (early season) than during 2000 (late season). This might be the main reason for the differences in root activity between the two years.

It was reported that nitrate-N application is more efficient than ammonium-N in increasing leaf chlorophyll content, soluble protein content, RNA and zeatin content as well as photosynthetic rate and grain yield [19]. In this study we found that nitrate-N application can also significantly improve root activity. Therefore nitrate-N application can improve both sink activity and source supply. Although the effect of respiration inhibitor NaN_3 on root activity and chalkiness was not consistent for the two varieties, the negative correlation between chalkiness and root activity during grain filling was consistent for both varieties.

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