*Spikelet dominance and sterility in rice (*Oryza sativa* L.) genotypes: a mechanism of survival?*

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ORIGINAL ARTICLE

Spikelet dominance and sterility in rice (*Oryza sativa* L.) genotypes: a mechanism of survival?

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Abstract Spikelet sterility is one of the poorly understood complex problems in rice and rarely harvest index exceeded 50 % in crop plants. Experiments were conducted on 800 rice cultures belonging to wide range, to identify genotypes that have low spikelet sterility, and N influence on selected genotypes, adapting a split plant technique approach to reduce sterility and removal of spikelets from panicles. Genotypes belonging to IR64 background (VG lines) and Tequing (TG lines) and other groups were screened. TG lines and japonica had greater percentage of filled grains and also relatively higher chaff content compared to that of VG lines. Application of nitrogen resulted in significant increase in secondary branch number, confirming that these were controlled by fertilizer application, while filling of spikelets on primary branches seems to be under genetic control. Evidence using retransplanting splitted transplanted seedlings after four tiller stage did not show significant yield reduction and harvest index. Spikelet removal technique from apical, middle and or basal regions for 1/3rd of the panicle, resulted in an increase in percentage filled grains, which was more when apical region spikelets were removed than at the basal region. In hybrids, the increase was as high as 20 %. Regression equations for all possible six combinations were developed for deriving a quadratic equations for (%) filled grain and grain weights separately for varieties and hybrids. Based on these experiments, 5–10 % in varieties and 10–20 % in hybrids spikelets are physiologically sterile. It will be possible to increase 5 % grain yield in rice by management and by breeding for rice yield improvement.

Keywords Screening · N application · Split plant · Drought contingency · Spikelet removal · Grain filling

Introduction

Breeding efforts to enhance yield are often dependent on the basic understanding of various physiological processes that are taking place within the plant (individual/genotype), surroundings (community/environment) or both interaction. Studies indicated that there was an increase in rice yields but the harvest index remains to be at 50 %. By taking advantage of specifically male spikelet sterility, hybrids were developed. In hybrids, the increase in the number of spikelets that enhanced grain yields mediated through growth process i.e. enhanced biomass production. Spikelet sterility and grain filling are two opposite but inter related phenomenon influencing rice yields. Factors responsible for partial grain filling and spikelet sterility in rice despite evolving modern cultivars and hybrids were found to be variable. In rice mature panicles, at a given time it is possible to find unopened, sterile (due to lack of ovules or anthers in case of hybrids), partially filled grains and filled grains. Grain filling studies on IET 1444 indicated 50–100 % spikelet removal induced secondary branch development. The spikelets flowered on 2nd day produced highest endosperm cells as well as weight, no compensation in late duration cultivars (Venkateswarlu et al. 1981) determined by inherent factors such as insufficient assimilate supply in Akenohoshi cultivar while in IR 65564-44-2-2 (New Plant Type) non fertilization (Sheehy

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et al. 2010; Kobata et al. 2006), it is the rate of accumulation not the long period of accumulation (Ansari et al. 2003) compact or loose panicle types (Wang et al. 2007), low light intensity (Voleti and Singh 1996) time of flowering, and hormonal levels which determine the percentage grain filling (Mohapatra et al. 1993; Patel and Mohapatra 1996). Many of these studies indicated a possibility of enhancing yield by physiological means but to what extent and the relevance of sterile spikelets not addressed.

Materials and methods

Four individual field experiments initiated from 2002 and continued up to 2006 are summarized here. About 813 crosses were screened for variation in spikelets for primary, secondary and chaff contents in 2002–2003 wet seasons, as shown in Table 1 were assessed. The second experiment was aimed at bringing the variation in sink size by application of nitrogen fertilizer on number of primary and secondary branches in selected genotypes viz., NDR 359, PA 6444 and Pusa Basmati. The third, virtue of the tiller producing ability of rice plants, in Vikas and DRRH-1 as one of the management option was analyzed. Vikas and DRRH-1 hybrids were transplanted in 1 m^2 area and left for normal growth. These plants when produced four tillers, two tillers were left behind (T1) while the other two tillers were split and re-transplanted (T2) in the field which was kept ready for transplantation. Similar procedure was adopted at eight tiller stage (T3—where in 6 tillers were left behind) and two tillers re-transplanted (T4) while traditional (undisturbed) planting served as control.

Table 1 Genetic variation in number of spikelets (range) on primary, secondary branches and sterile or unfilled spikelets at maturity in rice

Genotype	Primary	Secondary	Unfilled/chaff
VG (305)	8–76	5–110	3–130
TG (161)	20–219	17–232	4–132
IJDBK (5)	5–80	52–100	5–90
IJDA (26)	9–74	14–135	10–159
IJDB (16)	30–76	39–110	17–78
IJDC (38)	15–66	23–125	8–115
IJDD (27)	6–55	6–71	5–127
IJDE (12)	14–55	23–75	4–112
IJDJ (11)	25–100	40–134	2–146
IJDK (12)	49–88	58–151	6–149
IJDL (4)	50–81	81–104	20–98
Injav (110)	16–110	20–156	2–206
RWC (133)	15–82	5–152	1–97
PI54 (7)	19–48	33–70	3–50

Figures in parenthesis = number of lines from each group

VG introgression lines of IR 64 (BC2 F6), *TG* introgression lines tequing (BC2 F6), *IJDBK* IR 64 × HR (hara), *IJDA* N27 × LG, *IJDB* N27 × J7, *IJDC* N27 × Pusa 703-1-1, *IJDD* N27 × JV32, *IJDE* N27 × P3, *IJDJ* Savithri (SVR) × Longkayan (LG), *IJDK* SVR × Pusa 743 (PD), *IJDL* Temp.Jap (J7) × SVR, *Injav* Indica Japonica of exotic origin, *RWC* Restorer × WCV, *PI54* doubled haploid lines (IR64/Azaeucena)

The fourth experiment was on four each of the varieties, and hybrids grown with normal irrigation and cultural practices. There were four replications. Several number of fully opened panicles from the leaf sheath (emerged but no anthesis) on the same day were tagged. Spikelet removal treatment from apical (A), middle (M) and or basal (B) regions was imposed for 1/3rd of the panicle, while in control (C) set spikelets were fully intact. The panicles harvested were analyzed for fully filled grains and chaff using an automated grain counter (Contador, Pfeuffer). Grain weights were also recorded. Also, percentage grain filling values of varieties and hybrids at different spikelet removal treatments (apical—A, middle—M and basal—B regions) and with control (C) set spikelets were observed and relation among these variables was estimated by using regression equations.

Results and discussion

Genotypic variation in terms of spikelets on primary and secondary branches and chaff or unfilled grain of large set of germplasm is presented (Table 1). Though, the total number of spikelets per panicle varied from 25 to 300 only 31 % of crosses, have spikelets of primary nature between 51 and 75 while 25 % each recorded secondary and chaff. In general, Tequing lines had greater percentage of filled grains and also relatively higher chaff content compared to that of VG lines. Javonica lines had greater percentage of filled grains and this genetic background may be more suitable for genetic improvement of rice yield. The three genotypes, viz., NDR 359, PA 6444 (hybrid) and Pusa basmati grown under native N to different doses (50,100 and 200 kg/ha) was studied. Role of N nutrition on rice yields is well understood (Harrell and Blanche 2010) for achieving optimum yield but for the studies centered around other than spikelet sterility (Zhang and Yamaguchi 2010; Bond et al. 2008) except Ortega Blu (2007) and Kobata et al. (2010). Though it appears that, irrespective of treatments, yields did not vary as reported by Harrell and Blanche (2010), differences in terms of number of primary and secondary branches among the genotypes was evident (Table 2). It is evident that fertilizer application resulted in superior yields irrespective of genotype and the concomitant to primary and secondary branch number. Thus, stable number of primary branches (10–11) even under the influence of nitrogen treatment would not obviously result an increase in yield due to genetic control. On the contrary,

Table 2 Number of primary, secondary branches per panicle and grain yield under the influence of nitrogen (kg/ha) fertilizer

G/T	No. primary branches				No. secondary branches				Grain yield (g/m^2)			
	0	50	100	200	0	50	100	200	0	50	100	200
NDR 359	10.3	11	10.7	11.3	17	19	18	21	490	510	443	585
PA 6444	9.7	11	10.3	10	26.3	34.7	33	34.3	578	717	618	657
PB	10.3	10	10.3	11	28.3	26.7	28.7	32.3	560	450	455	482
CV (T)	9.6				30.4				38.1			
CV (g)	7.2				14.1				21.6			
CD main	0.4				3.2				88.9			
CD sub	0.4				2.1				68			

G genotype, *T* treatments, *PB* Pusa basmati

significant increase in the number of secondary branches (21–29) might be resultant consequence of N application i.e. external (environmental) factor. Thus, an interesting point that emerged from this leaves yet another pertinent question, are there two independent signals working in rice during grain filling process? Or is it because of non synchronization in spikelet opening that is governing the grain filling process. In the absence of biotechnological and modern facilities to dissect genetic signal, tiller producing capability of rice (Gravois and Helms 1992) though not 100 % satisfactory has been used as explained in materials and methods with an objective of achieving synchronization. Incidentally, this split-plant technique is normally being practiced in the eastern region of India specifically in an undulated terrain zones. This technique is refereed as split plant technique and the idea of this experiment was to observe that whether, the tillers so transplanted would produce similar yields or results in yield penalty due to availability of short time period to reach reproductive phase. Two genotypes, i.e. DRRH-1 hybrid and Vikas were characterized for biomass, yield and yield attributes under this study. To our surprise, the growth rates of tillers did not vary despite splitting and re- transplanting. Further more, all plants are headed normally as that of control set and the maximum difference between the treatments was observed only 4 days. Grain yield (g/m^2) did not show any significant differences between the treatments and genotypes, though, grain yield was more in Vikas compared to DRRH-1 (Table 3). Per cent chaff or sterile spikelets were more in DRRH-1 compared to Vikas and T4 had higher per cent chaff as compared to other treatments. Percent grains filled were higher in T2 in Vikas and T3 in DRRH-1 and G $\times$ T interaction was significant. Filled grains per panicle were more in Vikas compared to DRRH-1. Harvest index (HI%) was slightly higher in Vikas as compared to DRRH-1 though not significant with treatments. Between the two genotypes, Vikas was relatively superior by virtue of its biomass production and better partitioning ability compared to DRRH-1 hybrid. Hybrids are known to produce more number of spikelets and yet yield similarly as that of traditional varieties. This could happen when incomplete fertility restoration due to genetic factor or due to inefficiency in metabolite transfer to sinks resulting in lower percentage filled grains and or sterile spikelets. Under split plant technique non significant influence on various yield component factors could be seen that, invariably some amount of spikelet sterility persists in rice plants. Hastening tiller growth by adopting this split technique was to synchronize anthesis among different tillers which was successful. However, the data obtained on percentage filled grains and sterility has led to another interesting observation that, some amount of sterility is an essential evil like transpiration. Recently, Zhang and Yamaguchi (2010) have shown that downward regulation of a nearly constant amount of filled grains in four rice cultivars in spite of higher spikelet production.

This technique on the other hand, opened a possibility of growing rice crop for resilience as well as mitigation strategy under present climate change scenario. For

Table 3 Split plant treatment influence on grain yield, sterile spikelets and harvest index in hybrid DRRH-1 and variety Vikas

	GY (m^2)		Sterile spikelets (%)		HI (%)	
	DRRH-1	Vikas	DRRH-1	Vikas	DRRH-1	Vikas
Control	989	1,237	25.8	23.1	51.3	53.9
T1	896	1,034	28	17.7	46.2	50.9
T2	745	839	29.3	20	47.7	51.8
T3	721	748	25.3	18.5	50.4	49.0
T4	857	852	28.3	29.1	50.6	48.0
CD (ecotype)	NS		NS		NS	
CD (treatment)	NS		5.43		NS	
CD (g $\times$ t)	NS		4.7		NS	
S.E (g)	100		1.9		1.48	
S.E (t)	128		1.86		1.89	

For treatment details see Materials and methods section instance, under limited water availability till monsoon arrives, in a small area the crop can be grown normally till the plants reached four tiller stage i.e. 1 month time at the disposal of cultivator which can be used for preparation of remaining field or farm. Also, water required for irrigating rice, seed and one time application of basal fertilizer are conserved and used more efficiently. The strategy appears to be similar to that of SRI cultivation but can be as well adapted under heavy soil type conditions wherein operating cono weeder is rather difficult. Splitting plant perhaps, induced vigorous rooting in less time frame and also helps to maintain normal population density for better radiation use efficiency at later stages of re transplanting. Reduced land area at initial crop growth stages with more efficient use of fertilizers not only reduces the initial bursts of GHG emissions of both nitrous oxide and methane. Thus as a contingent plan the technique could be more useful and there is a need to screen and identify genotypes that produce tillers more rapidly particularly under drought.

Sterile spikelets are invariable has been evidenced as above, but to what extent panicle hold the grain weight and the sterility can be further reduced in a genotype? An attempt was made in this direction by removal of spikelets from different regions on a panicle at anthesis stage. Under the normal conditions, the percentage grain filling in varieties is more than that of hybrids (84.26 as against 73.65 %). Removal of spikelets from different regions improved the grain filling in both varieties as well as hybrids. However, the percentage increase in grain filling varied with the position of spikelet removal. Increase in percentage filled grains was more when the apical region spikelets were removed than at the basal region which is more conspicuous in case of varieties than hybrids (Table 4; Figs. 1, 2). The response varied from 12 % (Vikas) to 3 % when apical spikelets removed. In hybrids, the increase was as high as 20 % (Table 4). On the other hand, spikelet removal from middle region of the panicle resulted an increase of 5–15 % in varieties and hybrids. Though, an increase in percentage grain filling was recorded when the spikelets were removed from basal region it was lower than the remaining two treatments. Similarly, removal of primary branches caused an increase in grain filling by 6 % in varieties and 17 % in hybrids (Table 4). Also, the trend was similar in case of grain weight. Loss and compensations for both parameters were worked out based on the average of apical (A), middle (M) and basal (B) portions and verified with quadratic equation by developing a model for the purpose of predicting percentage grain filling and grain weight of varieties and hybrids at different spikelet removal and presented in Table 5.

In the present experiment sink capacity and source strength were tested for differences in rice varieties and hybrids so as to further relate that to what extent yield improvement is possible. Signal transmission indicators through fertilization for assimilate mobilization to source has been restricted by imposing treatment of spikelet removal before anthesis. Under the conditions of source not been disturbed, only one-third of the spikelets were removed from each of the zone at a given time, thus sink strength would not be limiting. Despite, this 100 % grain filling is not achieved though there was an increase in terms of percentage grain filling. Possibility of increase in percentage grain filling would be due to increased fertility as the main factor rather than source strength. Mean of the three treatments is more than that of actual percentage grain filling (i.e. control) and the difference between these would give rise to the possible increase. The opening of spikelets in a rice panicle had been reported to be 7 days duration from apical to basal regions (Kato et al. 2008) and this resulted in 5.4 % enhanced grain filling in varieties compared to that of 11.7 % in hybrids (Table 4). The actual chaff i.e. 100 % grain filling control value was relatively higher in hybrids (26.4 %) compared to that of varieties. Such large differences could arise under the

Table 4 Mean of percentage filled grains and thousand grain weight (g) in varieties and hybrids as influenced by removal of spikelets from different positions

Treatment	% Grain filling		1,000 Grain weight (g)	
	Varieties	Hybrids	Varieties	Hybrids
Control	84.26 (15.74)	73.65 (26.40)	20.87	20.24
Apical removed	91.12	86.92	21.28	20.36
Middle removed	89.69	83.35	21.23	20.18
Basal removed	88.20	85.78	21.32	19.46
CD (T)	4.71		0.74	
CD (g)	6.65		–	
S.Em (T)	1.6		0.25	
S.Em (g)	2.26			
Compensation	7.1	13.13	0.44	1.61
Possible grain yield	5.41	11.7		

Figures in parenthesis are percentage unfilled and chaff

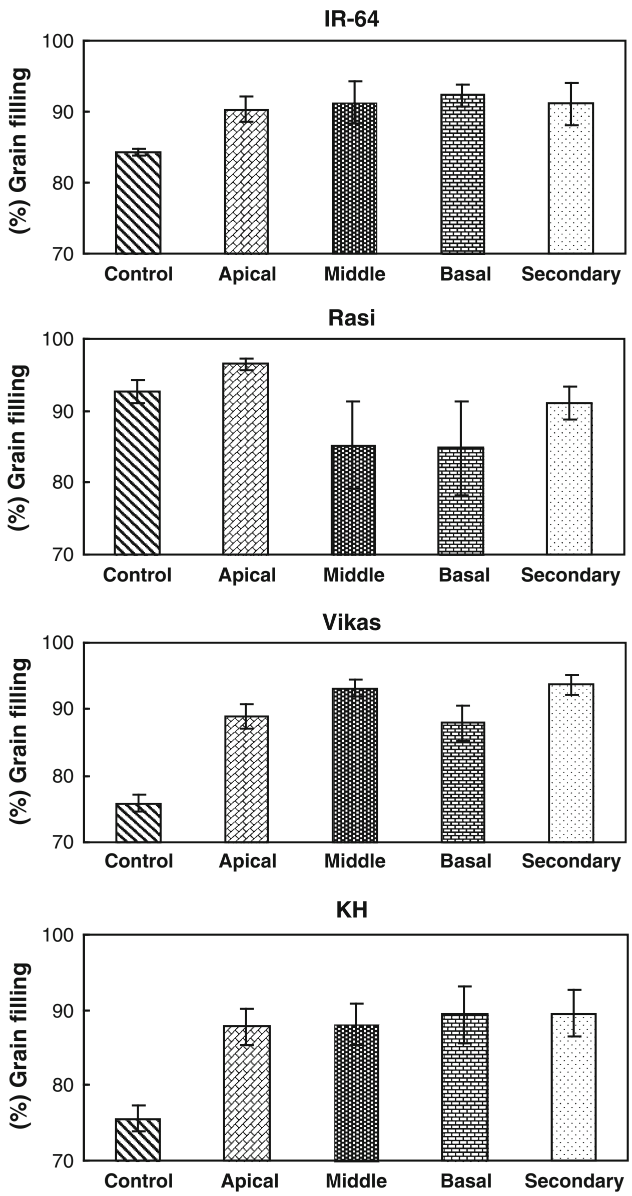

Fig. 1 Percent grain filling as influenced by spikelet removal from different regions in rice varieties

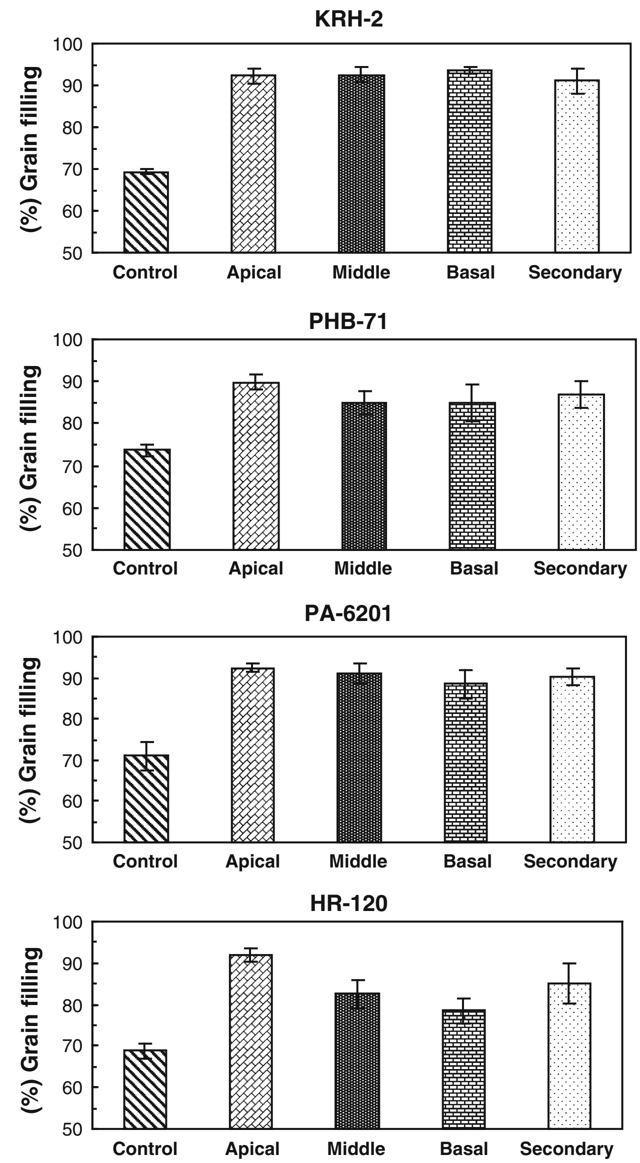

Fig. 2 Percent grain filling as influenced by spikelet removal from different regions in rice hybrids

conditions of incomplete restoration of fertility, ovule abortion and pollen infertility. This means in rice panicles, at least 10–15 % of unfilled spikelets would be inevitable and the significance of this event is discussed later. On the other hand, the second consequence of spikelet removal should result in better distribution of assimilate which should enhance the grain weights. The average grain weights of hybrids and varieties did not vary much (20.24 and 20.87 g respectively) while the possible increase was only 0.41 g in case of varieties. In hybrids there was no further improvement in grain weight as a result of removal of spikelets strongly indicating that source is a limiting

Table 5 Quadratic equations derived for varieties and hybrids by mathematical computations to predict percentage grain filling and grain weights from spikelet removal experiments for varieties and hybrids

	Varieties	Hybrids
% Grain filling	9.28 + 0.34A + 0.33B + 0.3M	106.4−0.135A−0.001B−0.117M
Grain weight	10.3 + 0.35A + 0.35B + 0.33M	0.24 + 0.37A + 0.36B + 0.33M

factor and ~12 % more grains could be filled in hybrids. On similar analogy, spikelet removal on primary branches resulted in 7.1 and 13.1 % in grain filling and 0.44 and 1.61 g in grain weights for varieties and hybrids respectively. These results indicate that, sink strength is relatively uniform in case of varieties irrespective of spikelet position whether it is on primary branch or secondary branch, where as in hybrids, it was the spikelets on the primary branch which exerts pressure for assimilate supply and or sink strength is greater than source strength (Directorate of Rice Research 2006, 2007). The second most important point that emerged from this study was, existence of hierarchy in grain filling pattern from apical to basal region, irrespective whether it is a hybrid or variety. This confirms all the earlier similar studies conducted by removing the spikelets (Khan and Choudhuri 1999; Kato 2004).

Models developed in this study, provided new and easy to use equations for the prediction of percentage grain filling and grain weight of varieties and hybrids at different spikelet removal treatments. However, working of model with variability in ripening rice cultivars is presently under investigation. Unfilled spikelets at the basal regions could be of physiological importance. Sequential opening of spikelets from apical to basal region results in providing elastic nature helping the panicle to overcome the breaking/bending of panicles during the grain filling process. The unfilled basal spikelets act as a cushion and support the panicle against the vagaries of wind. Also, incase of damaged apical spikelets either by wind or few grains consumed by birds or as shown in the present study by the spikelet removal studies, the basal spikelets comes to the rescue by way of grain filling which help the plant to produce some amount of seed for its own propagation. Thus, it could be a self adapted security measure by plant for its own propagation to overcome the adverse situations. Thus spatial and temporal relationship and physiological mechanism of grain filling process seems to be most important feature in understanding yield responses in rice. In essence these four experiments proved that grain filling below 90 % arises due to poor nutrition and management practices that are being followed during rice cultivation. In case of rice hybrids, another 5 % increase in grain filling and rice yields by breeding selected genotypes with low spikelet sterility would be a reasonable estimate.

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