Australian Journal of
Crop Science

AJCS

AJCS 12(08):1342-1350 (2018) ISSN:1835-2707
doi: 10.21475/ajcs.18.12.08.PNE1210

The within-panicle flowering sequence of hybrid rice affects seed vigour

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Abstract

Rice grains located along one panicle do not develop synchronously. Understanding of relationships between upper, middle and basal portions and flowering positions of rice panicles with grain weight, vigour and morphology is important for high-vigour seed production. In this study, four rice F_1 hybrids were used to analyse grain morphology, 1000-grain weight and seed vigour. The position of the seed on the panicle had a significant influence on seed vigour, with upper seeds having the highest vigour, followed by middle and basal seeds. The flowering sequence within a given branch affected seed size, 1000-grain weight and seed vigour, with differences in 1000-grain weight, mainly as a function of seed length. In each hybrid, the 1000-grain weight and vigour index of primary branches were superior to those of secondary branches. The flowering sequence of the primary grain branch was significantly correlated with 1000-grain weight and seed vigour index, with the highest values recorded at second and third flowering positions. In secondary branches bearing fewer than four grains, 1000-grain weight and seed vigour index exhibited a significant linear relationship with flowering sequence, in which the earliest maturing grains had the highest grain weight and vigour. When secondary branches included more than four grains, grain weight and vigour followed the same parabolic trend as in primary branches. Our findings indicate that 1000-grain weight and seed length can be used as indices of seed vigour.

Keywords: 1000-grain weight, flowering sequence, grain morphology, hybrid rice, seed vigour.

Introduction

Rice (*Oryza sativa* L.), a globally important food crop, is the basis of food security in many countries. In recent years, especially in developing countries, rice production has not matched the food demand of an increasing population (Chelliah and Gunathila, 2012). To meet this growing demand, rice production has to be raised by at least 70% over the next three decades (British Council, 2016). The land area devoted to rice cultivation is limited and production cannot be increased by increasing the acreage (British Council, 2016). Therefore, additional applied research is needed to find other ways to increase productivity. Seed quality, one of the most important factors influencing crop quality and yield, is a good indicator of the marketability (Milosevic et al., 2010) and potential performance of seeds in the field. Seed germination and vigour, which are components of seed quality, are thus important characteristics to be considered by farmers before planting (Finch-Savage et al., 2016).

Increasing the grain number per spike is an excellent way to improve rice grain yield potential. A rice spike is composed of spikelets that produce reproductive structures called florets. The grain number and grain weight of rice are influenced by both genetic and environmental factors (Huang Qing, 2014). These two parameters vary unevenly within a spike because of its unbalanced development (Miralles and Slafer, 1995; Chen and Yuan et al., 2007; Ferrante, 2015) and also depend on the spatial position of the grain (Calderini and Reynolds, 2000). In particular, these differences can be attributed to the poor filling of inferior spikelets due to carbon limitations (Murty, 1982; Dreccer et al., 2009; Wang, 1981; Hasegawa, 2017), sink capacity limitations (Kato, 2004), unbalanced hormone levels (Wang, 2015; Yang Zhang, 2006; Zhang Tan et al., 2006), low expression and activity of enzymes involved in sucrose-to-starch conversion (Ishimaru et al., 2005；Jeng et al., 2003; Wang et al., 2008) and impediments to assimilate transportation (Serrago et al., 2013; Yang and Zhang, 2010). Because the ability of developing grains to absorb nutrients differs according to their location on a given panicle, different branch portions and grain positions may cause variation in seed vigour, morphological parameters and grain weight, the latter closely related to grain filling (Fu, 2012). Differences in grain filling rates and final grain weight are mainly due to differences in flowering time (Hato, 1975). Early flowering is associated with strong assimilation ability and promotes rapid grain filling, thereby improving morphological parameters and grain weight. In contrast, late flowering delays grain filling, impedes assimilate acquisition, and frequently leads to inferior grain production and low seed-setting rates (Liu and Xie, 2001; Wang and Cheng, 2004; Yang et al., 1999). Some authors studies the different positions of rice grain weight and seed vigor research (Shi and Zhu, 2002) and also the effect of flowering sequence of spikelets on grain quality of rice. A preliminary analysis showed the effects of seed size and weight on seed vigor of hybrid rice (Liu and Xie, 2001) but the relationship between flowering sequence and seed vigour has not been reported yet.

In this study, we examined variation in grain morphological parameters, grain weight and seed vigour in primary and secondary branches and upper, middle and basal portions of panicles of four rice F_1 hybrids. The aim of our study was to provide a theoretical basis for development of a method for selection of high-vigour rice seed.

Results

Variation in grain morphological parameters

Hybrid rice grain morphology (length, width and thickness) and 1000-grain weight varied according to panicle and grain position in the four F_1 rice hybrids. Rice grain length and width were highest in upper and middle portions of panicles. Except for grain length in O3S/R1813, differences in grain length, width and thickness were not significant among different panicle positions. In all four F_1 hybrids, 1000-grain weight followed the trend of upper > middle > basal, with significant differences between upper and basal portions of panicles. Significant differences in 1000-grain weight were also observed between upper and middle portions of panicles of all F_1 hybrids except for O3S/R1813 (Table 1). The length, width and thicknesses of grains from primary branches were higher than those in secondary branches, but these differences were not significant according to the results of *t*-test (Table 2). Grain lengths were significantly different among different flowering positions of primary and secondary branches, but no significant differences were detected in grain width and thickness for most combinations. Grains at the second and third flowering positions of primary branches were longer than those at fourth, fifth and sixth positions. The order of grain lengths on secondary branches was consistent with the flowering sequence. Grains of O3S/R1813 were significantly longer than those in D8S/R1813, H638S/R1813 and Y58S/R1813. The mean 1000-grain weight per spike of the four hybrid rice varieties was generally higher in primary than secondary branches. In all four hybrids, a parabolic relationship was observed between grain weights of primary branches and flowering sequence, whereas the relationship between grain weights of secondary branches and flowering sequence was linear (Figure 2). The pattern of 1000-grain weight variation was consistent with that of grain length. The grain weight was decreased with flowering sequence. This trend varied slightly among different F_1 hybrids, with the fastest rate of change measured in the H638S/R1813 hybrid. According to *t*-test, the average 1000-grain weight of primary vs. secondary branches was not significantly different (Table 2). The highest grain weight was in O3S/R1813, which was consistent with grain length trend.

Vigour index

Seed vigour index in different panicle regions of the four hybrids F_1 followed the order of upper > middle > basal (Table 3). No significant difference in germination rate was detected among these three regions. In H638S/R1813 and ShenD8S/R1813 hybridsF1, seed germination potential, germination index and vigour index exhibited significant differences among upper, middle and basal panicle regions.

Except in O3S/R1813 hybrid rice, the seed vigour, germination rate and germination index of primary branch grains were higher than those of secondary branches. According to the *t*-test, vigour-index mean values were not significantly different between primary and secondary branches (Table 4). Significant differences were found in the germination index of primary branch grains at different flowering positions; whereas the same was true for secondary branches. No significant difference was detected in the germination potential and germination rate of most combinations. On primary branches, the germination indexes of seeds formed at second and third flowering position seeds were higher than those of fourth, fifth and sixth positions. On secondary branches, the trend in the seed germination index was consistent with the flowering sequence. Germination indexes of ShenD8S/R1813 and Y58S/R1813 were higher than those of O3S/R1813 and H638S/R1813. Grain numbers of primary and secondary branches differed among F_1 hybrids (Table 4). The relationship between the seed vigour index of primary branch grains and flowering position followed a parabolic curve (Figure 3). A large difference was observed in the number of grains (Table 4) within secondary branches of four hybrids. In secondary branches bearing three grains, the vigour index was highest at the first flowering position, followed by second and third positions; thus consistent with the flowering sequence. The relationship between seed vigour index and flowering position was linear in H638S/R1813 and Y58S/R1813 and parabolic in ShenD8S/R1813 and O3S/R1813 (Figure 3).

Correlations among different parameters

A correlation analysis was performed to examine relationships among 1000-grain weight, vigour and flowering position on primary and secondary branches of the four hybrid rice varieties (Table 5). Significant correlations were uncovered between grain weight and flowering position on both primary and secondary branches. This correlation was parabolic in primary branches and linear in secondary ones (Figure 2; Table 5). A parabolic correlation was found between seed vigour and flowering position on primary branches; whereas the same was true for seed vigour and flowering position on secondary branches in ShenD8S/R1813 and O3S/R1813. Seed vigour in H638S/R1813 and Y58S/R1813 was linearly correlated with flowering position on secondary branches (Figure 3; Table 5). Except in the Y58S/R1813 hybrid, grain weight and seed vigour were significantly and positively correlated within upper, middle and basal parts of panicles (Table 5). In each hybrid, 1000-grain weight and vigour were positively correlated, but no correlation was observed between these two parameters across different F_1 hybrids. Significant parabolic correlations were detected between both grain weight and seed vigour vs. flowering sequence on primary branches (Table 5). Except for ShenD8S/R1813 and O3S/R1813 the grain weight and seed vigour were linearly correlated with flowering sequence on secondary branches. A parabolic correlation was uncovered between grain length and seed vigour on primary branches at different flowering positions. No correlation was detected between grain length and vigour within upper, middle and basal parts of panicles and among different flowering positions of secondary branches. Except in O3S/R1813, grain length and seed vigour from grains at different flowering positions of primary branches were significantly correlated (Table 5). These findings indicate that 1000-grain weight and seed length can be used as indexes of seed vigour.

Table 1. Morphological parameters of grains from upper, middle and basal parts of panicles.

Hybrid		GL（mm）	GW（mm）	GT（mm）	TGW（g）
	Upper	9.40a	2.80a	2.00a	24.30a
H638S/R1813	Middle	9.23a	2.82a	1.97a	23.95b
	Basal	9.27a	2.76a	1.95a	23.05c
	Upper	8.53a	2.64a	1.80a	20.41a
ShenD8S/R1813	Middle	8.72a	2.62a	1.73a	19.99b
	Basal	8.63a	2.61a	1.79a	19.40c
	Upper	9.10a	2.51b	1.77a	20.70a
Y58S/R1813	Middle	9.04a	2.68a	1.78a	20.31b
	Basal	8.88a	2.58b	1.78a	20.12b
	Upper	9.74a	2.48a	1.99a	24.35a
O3S/R1813	Middle	9.60ab	2.48a	2.03a	24.09a
	Basal	9.42b	2.48a	1.90a	23.52b

In the four rice hybrids F_1, morphological parameters of grains from upper, middle and basal parts of panicles mean comparison. Means with the same letter are not significantly different according to Fisher's LSD test at P ≤ 0.05. GL, grain length; GW, grain width; GT, grain thickness; TGW, 1000-grain weight.

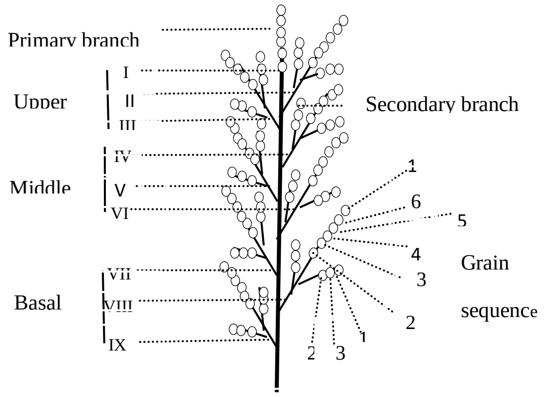

Fig 1. Schematic representation of a rice panicle.

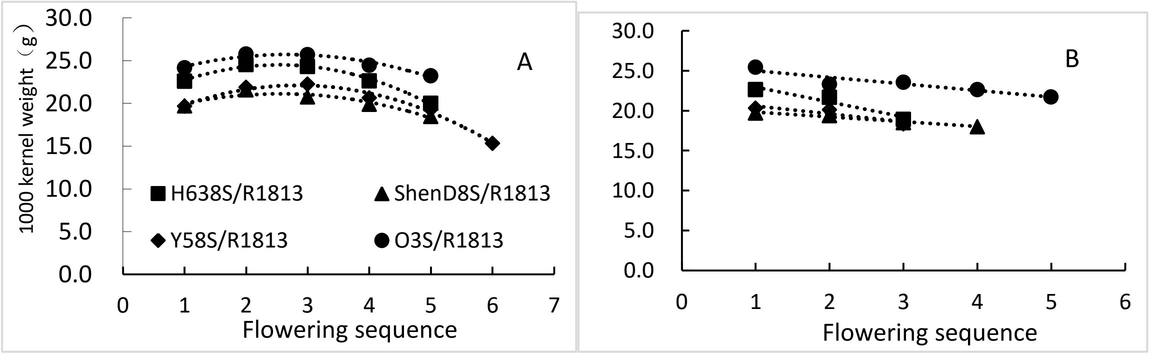

Fig 2. Relationship of 1000-grain weight and flowering sequence in four rice hybrids F1(A–B) .Relationship of 1000-grain weight and flowering sequence on panicle primary branches(A) and secondary branches (B).

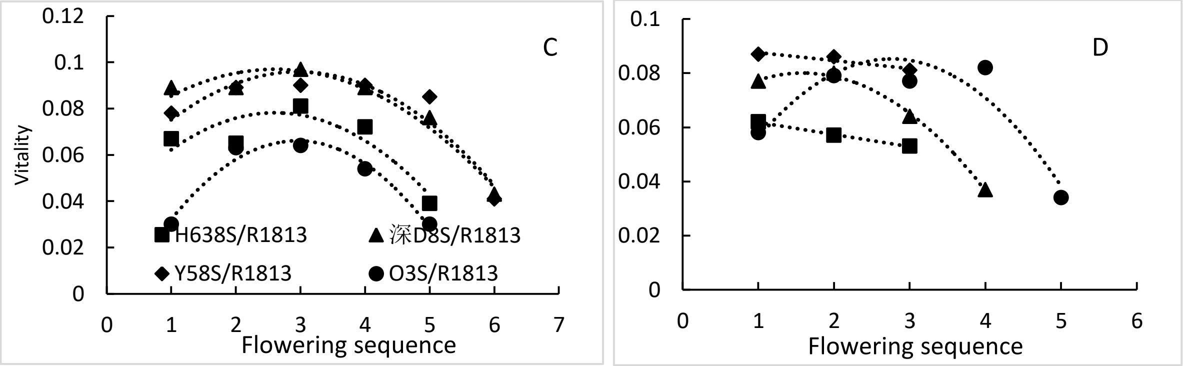

Fig 3. Relationship of seed vigour and flowering sequence in four F_1 rice hybrids. (C–D) Relationship of seed vigour and flowering sequence on panicle primary branches (C) and second branches (D).

Table 2. Morphological parameters of grains from different flowering sequence.

Hybrid			GL（mm）	GW（mm）	GT（mm）	TGW（g）
H638S/R1813	Primary branch sequence	1	9.62cd	2.82a	1.99a	22.59b
		2	9.86a	2.85a	2.01a	24.56a
		3	9.33ab	2.84a	2.00a	24.33a
		4	9.05bc	2.87a	1.90a	22.63b
		5	8.43d	2.82a	1.89a	20.01c
		Mean value	9.15	2.85	1.94	22.82
	Secondary branch sequence	1	9.42a	2.84a	1.89a	22.63a
		2	9.06a	2.76a	1.95a	21.68b
		3	8.34b	2.85a	1.94a	18.93c
		Mean value	8.94	2.82	1.93	21.08
ShenD8S/R1813	Primary branch sequence	1	8.82cd	2.63a	1.78a	19.73c
		2	9.22a	2.57a	1.81a	21.60a
		3	9.10ab	2.60a	1.84a	20.76b
		4	8.63d	2.66a	1.74a	19.89c
		5	8.39e	2.72a	1.73a	18.47d
		Mean value	8.85	2.64	1.79	19.68
	Secondary branch sequence	1	8.79a	2.69a	1.76b	19.72a
		2	8.45b	2.54b	1.81a	19.39b
		3	8.18c	2.62a	1.75b	18.53c
		4	7.84d	2.51b	1.73b	18.02d
		Mean value	8.32	2.59	1.76	18.92
Y58S/R1813	Primary branch sequence	1	8.90d	2.50c	1.75c	19.67d
		2	9.59a	2.67ab	1.81ab	21.88b
		3	9.38b	2.69ab	1.82a	22.26a
		4	9.12c	2.67ab	1.81ab	20.66c
		5	8.75e	2.70a	1.76bc	19.32e
		6	8.39f	2.61b	1.73c	15.32f
		Mean value	9.02	2.64	1.78	20.76
	Secondary branch sequence	1	9.15a	2.64a	1.79a	20.32a
		2	8.39b	2.67a	1.77a	20.12a
		3	8.39b	2.67a	1.77a	18.38b
		Mean value	8.64	2.66	1.78	19.61
O3S/R1813	Primary branch sequence	1	9.73b	2.55a	1.92a	24.17c
		2	10.13a	2.48a	1.98a	25.80a
		3	9.85ab	2.57a	1.96a	25.71a
		4	9.64bc	2.53a	1.94a	24.49b
		5	9.40c	2.56a	1.93a	23.22d
		Mean value	9.75	2.54	1.95	24.68
	Secondary branch sequence	1	9.92a	2.48a	1.96a	25.46a
		2	9.56b	2.36a	1.92a	23.32b
		3	9.18c	2.44a	1.93a	23.58b
		4	9.21c	2.43a	1.96a	22.65c
		5	8.88d	2.47a	1.82a	21.29d
		Mean value	9.35	2.43	1.92	23.26

In four F_1 rice hybrids, morphological parameters of grains from different flowering sequence. The mean value of the two stems. Means with the same letter are not significantly different according to Fisher's LSD test at $P \leq 0.05$. GL, grain length; GW, grain width; GT, grain thickness; TGW, 1000-grain weight.

Table 3. Seed vigour index of grains for the upper, middle and basal parts of panicles.

Hybrid		GR（%）	GP（%）	GI	VI
H638S/R1813	Upper	63a	39a	5.86a	0.063a
	Middle	64a	40a	5.96a	0.061a
	Basal	61a	30b	3.76b	0.033b
ShenD8S/R1813	Upper	97a	60a	8.95a	0.071a
	Middle	92a	57ab	8.53ab	0.067ab
	Basal	82a	46b	7.45b	0.060b
Y58S/R1813	Upper	97a	92a	11.53a	0.087a
	Middle	95a	93a	11.27ab	0.084a
	Basal	95a	88a	11.06b	0.079a
O3S/R1813	Upper	96a	89a	10.41a	0.097a
	Middle	89a	79a	9.78a	0.087a
	Basal	87a	81a	9.76a	0.073a

In four F_1 rice hybrids, seed vigour index of grains from upper, middle and basal parts of panicles mean comparison. Means with the same letter are not significantly different according to Fisher's LSD test at $P \leq 0.05$. GR, germination rate; GP, germination potential; GI, germination index; VI, vigour index.

Table 4. Seed vigour index of grains from different flowering sequence.

Hybrid			GR（%）	GP（%）	GI	VI
H638S/R1813	Primary branch sequence	1	69a	57ab	7.5a	0.067a
		2	63ab	50bc	7.1a	0.065a
		3	62ab	60a	7.5a	0.081a
		4	63ab	53abc	7.1a	0.072a
		5	54b	48c	5.0b	0.040b
		Mean value	62	54	6.8	0.065
	Secondary branch sequence	1	60a	49a	6.6a	0.062a
		2	57a	45a	6.2a	0.057a
		3	58a	54a	5.4a	0.053a
		Mean value	58	49	6.1	0.057
	T value		1.515	1.251	1.318	1.048
ShenD8S/R1813	Primary branch sequence	1	95a	73a	10.2a	0.089a
		2	93a	77a	10.1a	0.089a
		3	97a	79a	10.5a	0.097a
		4	95a	72a	10.1a	0.089a
		5	91a	69a	9.7a	0.076a
		6	94a	72a	6.0b	0.043b
		Mean value	94	74	9.4	0.081
	Secondary branch sequence	1	90ab	63a	9.4a	0.077a
		2	89ab	63a	9.4a	0.080a
		3	95a	59a	9.5a	0.089a
		4	84b	61a	5.1b	0.037a
		Mean value	90	62	8.4	0.071
	T value		1.941	6.843	0.842	0.695
Y58S/R1813	Primary branch sequence	1	95a	72a	10.1a	0.078b
		2	95a	77a	10.5a	0.089ab
		3	95a	82a	10.6a	0.09a
		4	91a	81a	10.4a	0.09a
		5	95a	85a	10.8a	0.085ab
		6	95a	86a	6.1b	0.041c
		Mean value	94	81	9.8	0.079
	Secondary branch sequence	1	96a	85a	10.8a	0.086a
		2	93a	83a	10.9a	0.089a
		3	94a	81a	10.7a	0.084a
		Mean value	94	83	10.8	0.086
	T value		0	-1.028	-1.422	-0.946
O3S/R1813	Primary branch sequence	1	93ab	31a	4.5c	0.030c
		2	90b	49a	8.4a	0.063a
		3	93ab	43a	8.5a	0.064a
		4	91ab	53a	6.9b	0.054b
		5	96a	41a	4.3c	0.030c
		Mean value	93	43	6.5	0.048
	Secondary branch sequence	1	93a	40b	8.4a	0.058b
		2	89a	69a	9.5a	0.079a
		3	93a	67a	9.4a	0.077a
		4	89a	71a	9.5a	0.082a
		5	87a	61a	4.9b	0.034c
		Mean value	90	62	8.3	0.066
	T value		1.518	-2.68	-1.433	-1.505

In four rice hybrids F_1, Seed vigour index of grains from different flowering sequence mean comparison. Means with the same letter are not significantly different according to Fisher's LSD test at $P = 0.05$. GR, germination rate; GP, germination potential; GI, germination index; VI, vigour index. Different lowercase letters in the same row indicate significant differences between one or more sequences ($t_{6,\ 0.025} = 2.447$; $t_{8,\ 0.025} = 2.306$).

Table 5. Results of correlation analyses of different rice grain parameters.

Treatment	H638S/R1813		ShenD8S/R1813		Y58S/R1813		O3S/R1813	
	Equation	Coefficient(r)	Equation	Coefficient(r)	Equation	Coefficient(r)	Equation	Coefficient(r)
A	$y=-0.7602x^2+3.8518x+19.633$	0.994**	$y=-0.2834x^2+1.3899x+19.114$	0.932*	$y=-0.6497x^2+3.7064x+16.784$	0.961**	$y=-0.4936x^2+2.641x+22.185$	0.970**
B	$y=-1.852x+24.786$	0.963*	$y=-0.596x+20.406$	0.968**	$y=-0.978x+21.551$	0.91	$y=-0.8156x+25.791$	0.932**
C	$y=-0.0064x^2+0.0336x+0.0244$	0.890*	$y=-0.0044x^2+0.0232x+0.0667$	0.979**	$y=-0.0053x^2+0.0317x+0.0487$	0.942**	$y=-0.0089x^2+0.0527x-0.0116$	0.982**
D	$y=-0.012x+0.0763$	0.948*	$y=-0.0136x+0.0985$	-0.896*	$y=-0.0015x+0.0883$	0.982*	$y=-0.0089x^2+0.0527x-0.0116$	0.982**
E	$y=0.0254x-0.5501$	0.966*	$y=0.0098x-0.1301$	0.995**	$y=0.0127x-0.1755$	0.942	$y=0.0282x-0.5501$	0.993**
F	$y=-0.0024x^2+0.1143x-1.2847$	0.928*	$y=-0.0057x^2+0.235x-2.3211$	0.983*	$y=-0.0125x^2+0.5321x-5.5634$	0.918*	$y=-0.001x^2-0.0344x+0.293$	0.910*
G	$y=0.0022x+0.0107$	0.944	$y=-0.0507x^2+1.9321x-18.296$	0.903	$y=0.003x+0.0258$	0.998**	$y=-0.0103x^2+0.4909x-5.7635$	0.932*
H	$y=-0.0741x+0.796$	0.986*	$y=-0.0277x+0.3047$	0.526	$y=0.0353x-0.2347$	0.994**	$y=0.0751x-0.6346$	0.999**
I	$y=-0.0464x^2+0.8638x-3.9473$	0.971**	$y=-0.0487x^2+0.8756x-3.8424$	0.925*	$y=-0.1539x^2+2.8701x-13.281$	0.956*	$y=-0.0216x^2+0.4674x-2.4572$	0.730
J	$y=0.0079x+0.0136$	0.978**	$y=-0.1264x^2+2.1355x-8.9326$	0.0897	$y=0.0046x+0.0449$	0.629	$y=-0.1479x^2+2.7989x-13.157$	0.976**
K	$y=2.8028x-3.1227$	0.852*	$y=3.3252x-9.2772$	0.950**	$y=3.5986x-12.163$	0.946**	$y=3.6891x-11.29$	0.914*
L	$y=3.4857x10.08$	0.997*	$y=1.9123x+3.0097$	0.972**	$y=1.4164x+7.3625$	0.582	$y=3.1678x-6.2746$	0.915

(A) 1000-grain weight vs. flowering sequence on primary branches; (B) 1000-grain weight vs. flowering sequence on secondary branches; (C) Seed vigour vs. flowering sequence on primary branches; (D) Seed vigour vs. flowering sequence on secondary branches; (E) Seed vigour vs. 1000-grain weight from upper, middle and basal parts of panicles; (F) Seed vigour vs. 1000-grain weight at different flowering sequences of primary branches; (G) Seed vigour vs. 1000-grain weight on secondary branches; (H) Seed vigour vs. grain length from upper, middle and basal parts of panicles; (I) Seed vigour vs. grain length from different flowering sequences of primary branches; (J) Seed vigour vs. grain length at different flowering sequences of secondary branches; (K) 1000-grain weight vs. grain length at different flowering sequences of primary branches; (L) 1000-grain weight vs. grain length at different flowering sequences of secondary branches.* and ** Level of significance at $p < 0.05$ and$p < 0.01$ respectively. $r_{0.05}$, 2 = 0.950; $r_{0.01}$, 2 = 0.990; $r_{0.05}$, 4 = 0.811; $r_{0.01}$, 4 = 0.917.

Discussion

The potential ability of a rice hybrid to exhibit increased yield, grain vigour and grain weight is affected by genetic and environmental factors (Cheng et al., 2007). Flowering position has a significant effect on seed vigour. The duration and sequence of grain filling in rice leads to different levels of grain plumpness in panicles. For example, rice grains from upper and middle portions are characterized by higher seed vigour than basal grains, and seed vigour is higher on primary than secondary branches. Grain filling in rice is a complex physiological process that is subject to significant gene–environment interactive effects (Lin et al., 2011). The quality of developing grains is also shaped by temporal and spatial differences in gene expression and regulation (Lin et al., 2011). A morphological analysis found that lower, inferior rice grains experienced a period of stagnation prior to the start of grain filling after fertilization (Ishimaru et al., 2003), whereas superior grains immediately entered a period of rapid accumulation of starch. Superior grains, which are located in the upper and middle parts of panicles, generally fill early and develop quickly (Lin et al., 2011). Unsynchronized grain filling is frequently observed in large-panicle-type and conventional hybrid rice varieties (Lin et al., 2011). Many studies have shown that the low 1000-grain weight and seed setting rate of inferior grains limit the high yield potential of rice. This result is mainly due to the asynchrony of grain filling between superior and inferior grains (Yang, 2010; Lin et al., 2011; Ishimaru et al., 2003, 2005; Huang and Liang, 2003; Huang, 2005). Grain weight can differ significantly among grain positions, not only in rice but also in crops such as wheat, maize and rapeseed (Gonzalez, 1993; Gao et al., 2001; Li and Shi, 2001; Shi and Zhu, 2002; Li, 2007). Within panicles, grain morphological parameters, grain weight and vigour were lowest at the base, with significant differences in seed vigour uncovered among lower, middle and upper parts of panicles. We uncovered a significant correlation between grain weight and vigour in different parts of the panicle, a result inconsistent with the observations of Lin et al., (2014). In terms of specific flowering positions, we found that grain morphological parameters, grain weight and seed vigour were higher in grains on primary than secondary branches, but these differences were not significant according to a *t*-test. The 1000-grain weight and vigour of grains from primary branches was parabolically correlated with flowering sequence. The shape of these relationship curves differed among the four F_1 rice hybrids, possibly because of differences in their grain-filling rates. Within primary branches, the seed germination index was highest at second and third flowering positions, followed by fourth, fifth and sixth positions. These results indicate that flowering sequence has a strong influence on seed vigour. The observed trends in 1000-grain weight in upper, middle and basal panicle portions and grain lengths of primary and secondary branches indicate that these two parameters can be used for seed grading. There is wide variation in significance of difference among traits of hybrids, in which not all the hybrids show similar significance results. This might be due to the genetic makeup differed between them.

In wheat, grain number and grain weight have been reported to differ greatly between spikelets according to their positions on the spike (Pan, et al., 2005; Pei, et al., 2008; HuiJuan, et al,. 2009). This positional variation also occurs in rice. For example, large differences have been observed between rice florets in terms of vascular bundle sizes and quantities, sink capacity, endogenous hormone levels, carbon amounts, activity and/or expression of enzymes involved in sucrose-to-starch conversion, and assimilate transportation, resulting in asynchronous grain development and nutrient accumulation as well as yield differences among spikelets and grains (Murty and Murty, 1982; Dreccer et al., 2009; Wang, 1981; Kato, 2004; Zhang and Tan et al., 2006; Ishimaru et al, 2005; Jeng et al, 2003; Wang et al, 2008). In rice, water loss in grains at the first flowering position of primary branches was more pronounced and occurred earlier than other grains, both before and after grain filling, which may partly be accounted for the low grain weight at the first grain position (Dong, 2011; Xue, 2014). Seed vigour is significantly correlated with the first flowering position of primary branches 1000-grain weight, which is one reason why seed vigour at the first flowering position was not the highest. The flowering sequence in rice has a significant effect on seed vigour. Within the same branch, the flowering sequence affects seed vigour, with differences in 1000-grain weight, mainly reflecting differences in grain length. Screening for high-vigour seeds on upper and middle parts of panicles can be based on 1000-grain weight. Because grain length and seed vigour at different flowering positions of primary branches are significantly correlated, low-vigour seeds on the upper and middle parts can be filtered out using a nest sieve. These results provide a theoretical basis for the screening of high-vigour seeds.

Materials and Methods

Seed material

We used *indica* rice sterile lines H638S, ShenD8S, Y58S and O3S as female parent, all crossed with the same male parent (R1813) to produce F_1 hybrids as experimental materials. Seeds of these materials were initially provided by the Yuan Longping Agricultural High Tech Co. (China).

Panicle morphology

Plants of the four sterile lines differed in terms of panicle branching patterns. Panicles of ShenD8S/R1813 and Y58S/R1813 were generally composed of 9 to 10 primary branches, with upper and basal portions each consisting of 3 primary branches and the middle portion comprising 3 to 4. In H638S/R1813 and O3S/R1813, panicles generally consisted of 10 to 11 primary branches; the upper and basal portions each included 3 to 4 primary branches, and the middle portion was composed of 4. To compare flowering sequence and grain characteristics, we labelled the five or six topmost flowering positions on primary branches and the three to four topmost positions on secondary branches according to the order of flowering as indicated in Figure1.

Measurements of morphological parameters and grain weight

Dry grains were collected from different flowering positions of primary and secondary branches and stored in small beakers labelled with the corresponding position until harvesting was completed (Figure 1). In this study, grain moisture content was measured using a near-infrared grain analyser, while grain length and width were measured automatically with a scanner. The thickness of 10 grains from each sample group was measured with a vernier caliper. The seeds were weighed on a F50-11-type electronic balance. After counting the number of grains, 1000-grain weight was then calculated.

Seedling growth test

Seedling growth tests were conducted according to the GB3543-1995 standard (Chinese,1995). After sowing seeds on sand at 25°C in a seed germination chamber, the number of germinating seedlings was recorded daily for 10 days. Lengths of seedlings (portions above root nodes) and maximum root lengths were recorded. After heating in an envelope in an oven (Shanghai Science Instrument Co., Shanghai, China) for half an hour at 105°C, the seedlings and roots were kept at 80°C until constant weight. Seed germination potential was calculated as the number of seeds germinating normally within the first 4 days/number of seeds tested for germination × 100. Germination rate was calculated as the number of seeds germinating normally within the first 4 days/number of seeds tested for germination × 100. Germination index (GI) was calculated according to the formula $GI = \Sigma Gt / At$, where Gt is the number of seeds germinated after 10 days and At is the number of days (Zhang and Wang 2006). Vigour index (VI) was calculated according to the formula $VI = GI \times S$, where S is seedling dry weight (Zhang and Wang 2006).

Experimental design and statistical analysis

All statistical analyses were conducted in IBM SPSS version 19. Data were analysed by one-way ANOVA (complete randomised design). Fisher's least significant difference test ($P \leq 0.05$) was used to detect significant differences between treatment means. A one-sample *t*-test was used as indicated.

Conclusion

The flowering sequence in rice has a significant effect on seed vigour. Within the same branch, the flowering sequence affects grain morphological parameters, 1000-grain weight and seed vigour, with differences in 1000-grain weight mainly reflecting differences in grain length. Screening for high-vigour seeds on upper and middle parts of panicles can be based on 1000-grain weight. Because grain length and seed vigour at different flowering positions of primary branches are significantly correlated, low-vigour seeds on the upper and middle parts can be filtered out using a nest sieve. These results provide a theoretical basis for the screening of igh-vigour seeds.

Acknowledgements

This study was financially supported by a Commonweal Industry (Agriculture) Science and Technology Project grant (201303005).

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