



# The influence of plant spacing in the early stages of selection of rice (*Oryza sativa* L.) varieties

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## Abstract

The cultural practices of the early generations in a pedigree breeding programme may influence its success. The main objective of this study was to compare two selection environments in rice: Widely spaced planting in the field and dense planting in concrete basins. Both methods had yielded commercial varieties in the past. Two F<sub>2</sub> populations (J and MS), derived from two crosses sharing the same female parent, were transplanted to both environments. Phenotypic traits were evaluated and their narrow sense heritabilities (h<sup>2</sup>) estimated in the F<sub>3</sub> and in the F<sub>4</sub> progenies of selected plants, all grown in the field. Growth potential was more apparent in the field for most traits, especially those related to yield, but broad sense heritabilities were higher in the basins for ten traits, being higher in the field for the other five. In population F<sub>2</sub>MS, field selection resulted in F<sub>3</sub> plants which retained a higher tillering ability than those derived from basins selection. Most traits showed low h<sup>2</sup> values: Additive variance was only relevant in panicle length (in both populations), plant height and mean panicle weight (in the J population). However, response to one generation of selection (from F<sub>3</sub> to F<sub>4</sub>) also showed fixable variation in panicle number. In addition, this selection reduced plant height, increased culm diameter and internode length (in both populations), and improved pulling resistance (against lodging) in population J. It may be concluded that both practices can be used for selection in the F<sub>2</sub>, although different responses might be expected in yield related traits.

**Additional key words:** lodging; heritability; pedigree breeding.

**Abbreviations used:** CD (culm diameter); FL (flag leaf length); G×E (genotype × environment interaction); GH (growth habit); GL (grain length); GS (grain shape); GW (grain width); IL (second basal internode length); IVIA (Instituto Valenciano de Investigaciones Agrarias); J ((Z9 × Leda)–2 × JSendra); MAT (days to maturity); MS ((Z9 × Leda)–2 × (Marjal × Senia)–16B); PH (plant height); PL (panicle length); PN (panicle number); PR (Pulling resistance); PW (average panicle weight); QTL (quantitative trait loci); SL (stem length); TW (total panicle weight); WP (percent of grains with white core); WS (mean white core size).

**Parameters:** h<sup>2</sup> (narrow sense heritability); H<sup>2</sup> (broad sense heritability); Va (additive variance); Ve (environmental variance); Vg (genetic variance); Vp (phenotypic variance); D (additive contributions to genetic variation); H (dominance contributions to genetic variation); R (response to selection); S (selection differential); ρ (selection pressure).

**Authors' contributions:** Conceived and designed the experiments: AGY and PB. Performed the experiments: IT. Analyzed the data: AGY, IT and PB. Contributed reagents/materials/analysis tools: AGY and PB. Wrote the paper: AGY and PB.

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## Introduction

Rice (*Oryza sativa* L.) breeding, even if conducted locally, may have a broader impact, because the grain yield of flooded rice under conventional cultivation does not present much significant difference in varietal ranking (genotype × environment interaction, G×E)

within latitudinal ranges, if there are no biotic (pests or disease damages) nor abiotic (drought, salinity, toxicity or lack of oligoelements) stresses. There are multiple examples: the Italian variety ‘Balilla’ (which was semi-dwarf and productive before the Green Revolution) was cultivated in the middle of the 20th century both in Italy and throughout Spain. The semi-

dwarf rice varieties of the green revolution were cultivated in millions of hectares over Asia. Two Spanish varieties obtained from our Rice Department (now in IVIA) at the end of the 1960s were cultivated not only throughout Spain, but ‘Bahía’ also in Italy (with the name of ‘Padano’) and Australia, while ‘Sequial’ was also cultivated in Greece (with the name of ‘Hispaniki’). In the 1980s, two Californian varieties were widely cultivated in Spain and Italy: L202 (called ‘Thaibonnet’ in Spain) and M202 (called ‘Thainato’ in Spain). The Australian long-grained variety ‘Doongara’ corresponds likely to ‘Puntal’ in Spain, where it is still the rice variety most widely cultivated.

However, the cultivation practices in the early stages of selection may influence the success of rice breeding. It is often assumed that single plant selection is not very effective to identify genotypes that will perform well in dense stands. Therefore, after the first breeding generations of a cereals’ pedigree breeding program, field plots are established when there is a reasonable uniformity of plant height, maturity and type, which is achieved in the  $F_5$  or  $F_6$  (depending on the cross). Selection in the first generations is essential to reduce the number of lines to be tested in the field, and single plant selection is mandatory because each individual has a different genotype. Nonetheless, if there are many paircrosses in one breeding program, selection can start by discarding whole  $F_2$  populations that perform poorly. Inside each  $F_2$  population, some rice breeders select plants transplanted under wide spacing, while others do so under narrow spacing. Jennings *et al.* (1979) warn that in the  $F_2$  of a cross between a tall parent and a short parent, if plants are narrowly spaced, competence is strong, and among other constrains, tall plants overshadow short plants; but if plants are widely spaced, it is difficult to distinguish short genotypes, because there is no competition for light. Such short stature is, usually, a desirable attribute as it contributes to reduce lodging (Khush, 1999). Fasoulas (1988) claimed that wide spacing selection for yield in  $F_2$  is more effective than narrow spacing, because plants display more their genetic potential. Ntanos & Roupakias (2003) obtained some rice varieties with this wide-spacing selection method (“honeycomb selection”).

Grain yield is a complex trait normally not selected in  $F_2$ , because its heritability is low in a population of individual plants at this early stage of selfing (Kearsey & Pooney, 1996). The same happens with lodging resistance, a trait that is often visually scored when lines are sufficiently homogeneous. An alternative is to measure some morphological or structural variables that are related to lodging; among those, authors highlight plant height, weight of the aerial part (particu-

larly that of panicles), or the strength or sturdiness of the stem (Seko, 1962; Xiao *et al.*, 2002; Kashiwagi & Ishimaru, 2004). In addition we included in this study some traits (internode length, culm diameter, and pulling resistance) to determine if they could be screened easily in early generations and be reliable to assess the genotypes’ tendency to lodge.

The objectives of this work were: (1) to compare two selection environments for the  $F_2$ : basins under narrow spacing, and field plots under wide spacing; (2) to determine the relative influence of genotype and environment on different characters in two crosses sharing the same female parent; and (3) to analyse the heritability and response to selection of lodging-related traits.

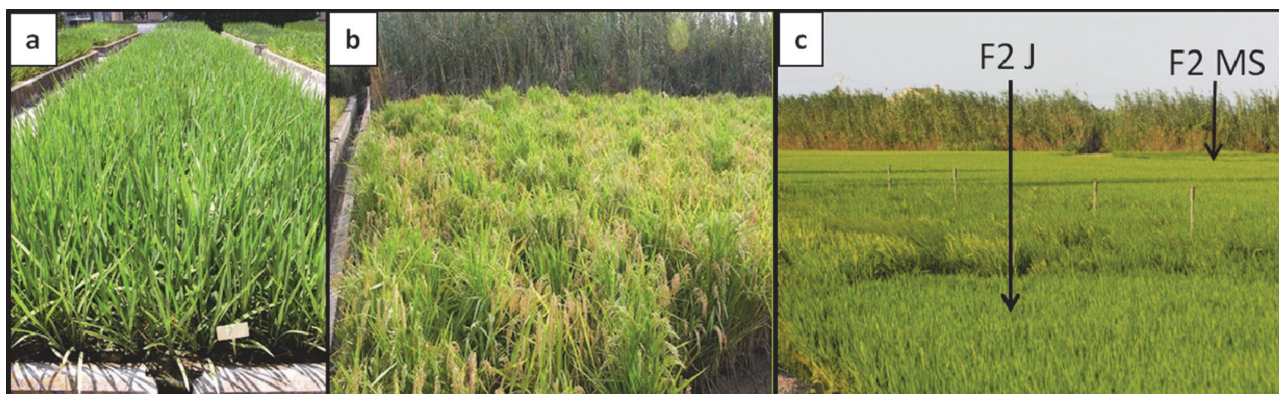
## Material and methods

### Plant material

The two populations were derived from crosses sharing the same female parent: ( $Z9 \times Leda$ )–2, a breeding line developed at IVIA, where ‘Z9’ is IRRI’s (International Rice Research Institute) breeding line IR66155-2-1-1-2 and ‘Leda’ is a variety obtained in our IVIA Department. Populations are named following the male parent: J is ( $Z9 \times Leda$ )–2  $\times$  JSendra (where ‘JSendra’ is a variety obtained in our Department); and population MS is ( $Z9 \times Leda$ )–2  $\times$  (Marjal  $\times$  Senia)–16B (where the male parent is also a breeding line from IVIA).

The  $F_1$  from each cross was selfed to obtain the  $F_2$ . In 2004, during the regular growing season in Valencia, more than 1450 plants of each  $F_2$  population were transplanted to concrete basins in rows, under a high plant density: 15 cm between rows and 10.5 cm between plants. A minimum of 1000 plants per population were transplanted to the field as well, but at a wider spacing of 50 cm between rows and 15 cm between plants. Within these populations, only 150 plants from each  $F_2$  could be evaluated (as they were used for a QTL study as well): 75 in the field and 75 in basins. In both cases, the soil was a clay-loam, with typical cultural practices for the region, but the  $F_2$  populations were transplanted to separate fields and basins (Fig. 1). Plants from variety ‘Senia’ (also from IVIA) were used as a check in the four combinations population/environment, to estimate the environmental variance of each combination, because this variety is one of the checks used in our breeding program, and an ancestor of both populations.

Each of the 150  $F_2$  plants per cross gave rise to an  $F_3$  family by natural selfing. In 2005, 30 plants per line (family) of all the 300  $F_3$ , plus the parents, were grown in the field, in rows, spaced at 15 cm  $\times$  50 cm. Every



**Figure 1.** Basins and field setting for  $F_2$  evaluation and selection. a)  $F_2$  under narrow spacing in a concrete basin; b)  $F_2MS$  under wide spacing in the field; c) relative situation of field plots used for  $F_2J$  and  $F_2MS$ .

25 lines, a row of ‘Senia’ was inserted. The seed of 6 random plants per  $F_3$  line was collected; the selected  $F_4$  were grown the following year in the same field conditions.

## Evaluation

In each  $F_2$  plant, and in 6 plants/line in  $F_3$ ,  $F_4$ , checks and parents, the following traits were evaluated: days to maturity (MAT) as the number of days from seeding to 95% of mature grains; plant height (PH) to top of the panicle; panicle length (PL) and culm diameter (CD) at the second basal internode, measured in 5 culms/plant; average stem length ( $SL = PH - PL$ ); growth habit (GH), visually scored with a scale of 1 (erect) to 6 (prostrated); panicle number (PN); total panicle weight (TW); average panicle weight ( $PW = TW/PN$ ).

In the  $F_3$ , days to maturity and growth habit were scored only once per row, since families were homogeneous. Flag leaf length (FL, in 4 culms/plant), 100 grains’ weight and grain measurements were only evaluated in the  $F_2$ .

Grain measurements were determined in 30 dehusked (whole) grains per plant: length (GL), width (GW) and their ratio (GS) were scored with an image analyser associated to Inspector programme (from Matrox); percent of grains with a white core (WP) and its mean size (WS) were visually scored; WS was calculated with the following index:  $(3 \times N^\circ \text{ of grains with big cores} + 2 \times N^\circ \text{ of grains with medium cores} + N^\circ \text{ of grains with small cores}) / \text{Total } N^\circ \text{ of white-cored grains}$ .

Lodging resistance traits were measured only in  $F_3$  and  $F_4$ : pulling resistance (PR) as is described in Torr o *et al.* (2011); second basal internode length (IL) of two culms per plant (because shorter internodes there could increase lodging resistance).

## Statistical analysis

Analyses of trait distributions and means, as well as ANOVA and correlation between traits, were performed in all populations, generations and genotype/environment combination, with the statistical packages: Statgraphics Plus 5.0® (Statistical Graphics corp. 1994-2000) and SAS 9.1 (SAS Inst. Inc., Cary, NC, USA). The ANOVAs made for comparison between environments and between populations had one factor (between groups) and the error term (within groups). Some traits were transformed to standardize their distribution: PN and PR were transformed by the logarithm, CD in  $F_3$  was transformed by the inverse ( $1/x$ ), and WP by the arc sin of its square root (Bliss’ transformation, to compress the tails of this typically binomial distribution). As mentioned, MAT and GH in  $F_3$  had only one score per line, and consequently narrow sense heritability was not estimated. For multivariate analyses (principal components analysis), only the traits not correlated (data not shown) were considered (for example, we did not include stem length, as it is highly correlated with plant height).

## Genetic analysis

Heritabilities were calculated by analyses of variance. Using the notation of population genetics,  $V_p$  (phenotypic variance of a trait in a population) =  $V_g + V_e$ , where  $V_g$  is its genetic variance and  $V_e$  its environmental variance. The broad-sense heritability is the genetic proportion of its total variation, and is thus estimated as:  $H^2 = V_g/V_p$ .

In  $F_{2s}$ , only  $H^2$  could be calculated:  $V_g = V_{pl} - V_e$ , where  $V_{pl}$  is the variance between population plants and  $V_e$  (environmental variance) is the variance between homozygote check plants. In traits with more than one measurement per plant, the analysis is one case of the general ANOVA (Table 1).

There is only one family in an F<sub>2</sub>:  $f = 1$ .

$V_e = V_{e1} + V_{e2}$ , where  $V_{e1}$  is the variance of plant means ( $\sigma^2_{pl}$ ) in a homozygous check.  $V_{e2}$  is the variance within plants ( $\sigma^2$ ).

$$V_g = \sigma^2_{pl} - V_{e1}.$$

The error of H<sup>2</sup> was estimated as the significance of  $V_p/V_e$  (Kearsey & Pooney, 1996).

From the analyses of variance of F<sub>3</sub>, both H<sup>2</sup> and narrow-sense heritability ( $h^2$ ) can be calculated:

$V_g = V_a + V_d$ , where  $V_a$  is the additive genetic variance (the variation due to gene effects fixable in homozygosis) and  $V_d$  is the dominant genetic variance.

$$h^2 = V_a/V_p.$$

Using Mather's notation of self-compatible crosses,  $V_a = (1/2)D$  and  $V_d = (1/4)H$ , where  $D$  represents the contribution of additive genetic effects and  $H$  represents dominance contributions.

The ANOVA is another case of Table 1, where the within families variance is:  $\sigma^2_{pl} = (1/4)D + (1/8)H + E1$ , being  $E1$  the variance of the specific environment, that is the environmental variance between plants within families (estimated by  $\sigma^2_{pl}$  in the homozygote check [variance within rows], in which  $D = H = 0$ ).

$\sigma^2_B = (1/2)D + (1/16)H + E2$ , where  $E2$  is the variance of the common environment, the environmental variance between families (estimated by  $\sigma^2_B$  in the homozygote check).

In F<sub>3</sub> traits with more than one measurement per plant, Table 1 applies fully. Here:  $\sigma^2_{pl}$  is the variance of plant means within families;  $\sigma^2$  is the error term ( $E3$ );  $V_e = E1 + E2 + E3$ ;  $\sigma^2_{pl} = (3/32)H + E1 - (1/2)E2 + (1/2)\sigma^2_B$ ;  $\sigma^2_B$ ,  $E1$  and  $E2$  are the same estimators as described for traits with one measurement per plant (Chandraratna, 1964).

## Selection

Two selection processes were carried out independently: the complete F<sub>2</sub> populations underwent selection as part of our breeding programme ("global selection"), taking into account mainly PH, MAT, PN and TW,

however PL was also measured. A 2% selection pressure was applied in basins (30 plants were selected in population MS, and 29 in population J), while in the field, the selection pressure was 1% (11 and 8 plants selected in MS and J, respectively). Due to lack of space, the F<sub>3</sub>J families derived from the field could not be grown; as for the MS cross, we analysed 3 and 14 F<sub>3</sub> families derived from F<sub>2</sub> mother plants selected in the field and in basins, respectively. The derived F<sub>3</sub> lines were transplanted at wide spacing (30 cm × 30 cm) in basins for further selection as is the practice in our Department.

In the 300 unselected F<sub>3</sub> lines (derived from the 75 F<sub>2</sub> plants of each genotype/environment combination) grown in the field, traits related to lodging (PH, CD, PN, IL and PR) were selected ("Lodging selection") using a selection pressure (proportion of selected plants:  $\rho$ ) of 5% between families and of 1/6 within families. It resulted in 7-8 lines being selected per cross with the extreme values in each of the abovementioned traits; and, within the selected lines, seeds from the plant with the extreme value were grown as F<sub>4</sub> the following season. CD and PR were positively selected, PH was negatively selected, and for the PN and IL a bidirectional selection was used. Selection differential ( $S$ ) was calculated as the difference between the mean of selected F<sub>3</sub> lines ( $M_{selec}$ ) and the overall F<sub>3</sub> mean ( $M_{F3}$ ); response to selection ( $R$ ) was calculated as the difference between the mean of the F<sub>4</sub> lines derived from selected F<sub>3</sub> lines ( $M_{F4}$ ) and  $M_{F3}$ , checking its significance with the Student  $t$  test. Since both generations were grown in the same field, but on consecutive years, values were corrected with the check 'Senia' for environmental effects;  $R$  is, therefore, only a rough estimate of response to selection.

## Results

### Preliminary results

Since 1991 we have selected F<sub>2</sub> in the field (wide spacing) and in basins (narrow spacing). As a result, nine varieties were commercially released until 2014. Four of these crosses had their F<sub>2</sub> selected in both environments: Three of their resulting varieties ('Gavina', 'Cormorán' and 'Sivert') came from an F<sub>2</sub> mother plant selected in basins, and the fourth ('JSendra') had its F<sub>2</sub> mother plant selected in the field. Four other varieties ('Albufera', 'Argila', 'Antara' and 'Fleixa') came from crosses whose F<sub>2</sub> were only selected in basins. One variety ('Sarcet') came from a cross whose F<sub>2</sub> was only selected in the field.

**Table 1.** General analysis of variance of F<sub>2</sub> and F<sub>3</sub>

Source of variation	df	Expected mean squares
Between families	$f-1$	$\sigma^2 + m \sigma^2_{pl} + p \sigma^2_B$
Between plants (V <sub>pl</sub> )	$f(p-1)$	$\sigma^2 + m \sigma^2_{pl}$
Within plants	$fp(m-1)$	$\sigma^2$
Total (V <sub>p</sub> )	$fpm-1$	$\sigma^2 + \sigma^2_{pl} + \sigma^2_B$

$f$ : N° of families;  $p$ : N° of plants/family;  $m$ : N° of observations/plant;  $\sigma^2_B$ : variance of family means.

## Experimental results

Prior to the analysis of the different genotype/environment combinations, a comparison of environments was performed by ANOVA of the corresponding checks. In Table 2, contrast significances shadowed in the E, Pf and Pb columns indicate that differences between checks are significant, showing environmental differences between basins and fields, between fields or between basins. The two basins used in the F<sub>2</sub> trial were homogeneous for most studied traits (except FL, MAT and some grain traits), while many differences were found between the two field plots where the F<sub>2</sub> were grown. On the contrary, the field plots used for the F<sub>3</sub> (adjacent, not separate like the F<sub>2</sub> field plots) proved to be more suitable for comparison of the populations' performance, since only the GW of the checks varied significantly between them.

Table 2 shows that many traits expressed higher mean values in the F<sub>2</sub> plants grown in the field under wide spacing than in basins under narrow spacing, but in some cases differences were small. The most affected traits were those related to grain yield: panicle number and total grain weight showed a significant increase when cultivated in the field with respect to culture in basins (duplicating or even quadruplicating the values), especially in population J. The increase of panicle weight was not so marked. Culms were also significantly thicker in the field. An exception was flag leaf length: It was longer in basins than in the field. Plants showed a less open growth habit in the field.

In Table 2, contrast significances underlined in the E, Pf and Pb columns, indicate that differences between environments or between populations are significantly bigger than differences between checks, and so they can be attributed to genetic or G×E effects. Plant height and culm length showed G×E interaction: In the field, J plants were taller on average than in basins, while MS plants were shorter on average than in basins.

Plants were on average more productive and tillered more in F<sub>2</sub>J than in F<sub>2</sub>MS and F<sub>3</sub>J. As a mean, the F<sub>3</sub> plants tillered less than F<sub>2</sub> plants grown in the field. Perhaps as a consequence, culm diameter was bigger, although part of this difference was due to the different way of measuring the trait. In both generations, MS plants had thicker tillers than J plants.

The trait means of both populations in the field differed more in the F<sub>2</sub> than in the F<sub>3</sub>, since only PL, MAT, GH and CD showed significant differences between the F<sub>3</sub>; the two traits measured only in F<sub>3</sub> (IL, PR) also proved to be different. F<sub>3</sub>J had longer internodes and shorter panicles, matured later, had narrower culms, its plants were more open and presented higher pulling resistance than F<sub>3</sub>MS.

In the F<sub>2</sub>, broad sense heritabilities (H<sup>2</sup>) of plant height, panicle length, flag leaf length, 100-grain weight and mean grain-chalk size were higher in basins than in the field. But the H<sup>2</sup> of culm diameter, mean panicle weight and growth habit were higher in the field. Concerning total panicle weight, the only heritability (broad or narrow) different from zero was that of F<sub>2</sub>J in the field, but it was not significant, according to the F<sub>test</sub> (V<sub>p</sub>/V<sub>e</sub>=1.68). On the other hand, broad sense heritabilities were higher in F<sub>3</sub> than in F<sub>2</sub>, except for plant height, mean panicle weight and growth habit. Narrow sense heritabilities of many traits were low, *i.e.*, additive variance had little or no weight in determining them. However, it was high in panicle length (in both populations), and in the plant height and mean panicle weight of F<sub>3</sub>J (the latter only relatively high).

The highest broad sense heritabilities in the F<sub>2</sub> are those of elongation related traits, days to maturity, growth habit, grain dimensions and weight; pulling resistance and presence of white core are also noteworthy.

In the multivariate analysis of plant character means in both populations and both environments (Table 3), only the first three principal components (orthogonal— independent— linear combinations of characters) were informative. The first two principal components explained 80% of the variance of the variables' correlation matrix. The first principal component includes mainly panicle number, total panicle weight, growth habit, culm diameter and flag leaf length. The second includes mainly panicle length, grain length and width, and days to 95% maturity. The third includes mainly plant height, one-hundred grain weight, chalk percentage and size. Figure 2 represents the four combinations population/environment against the first two principal components. Populations grown in basins appear in the negative part of the first principal component. When grown in the field, they appear in the positive side. This confirms the effect of the environment mentioned before: plants in basins tillered less on average, with more slender culms, lower grain production, a more open growth habit and longer flag leaves than in the field. In both environments, J population appears in the negative part of the second principal component, while MS population appears in the positive side. This shows the following genetic effect: Plants of MS population matured earlier than those of J, while their panicles and grains were longer.

## Global selection

In order to test the suitability of both environments for selection, we compared F<sub>3</sub> plants derived from F<sub>2</sub>

**Table 2.** Mean, standard deviation (SD), broad sense (H<sup>2</sup>) and narrow sense (h<sup>2</sup>) heritability of plant traits in populations (Popl.) J and MS, in two environments: field and basins.

Character	Popl.	Field			Basins		ANOVA <sup>[1]</sup>		
		Mean ± SD	H <sup>2</sup>	h <sup>2</sup>	Mean±SD	H <sup>2</sup>	E	Pf	Pb
Plant height (PH, cm)	F <sub>2</sub> J	89.5 ± 8.0	0.80	–	84.8 ± 7.9	0.94	****		
	F <sub>2</sub> MS	84.5 ± 8.8	0.80	–	90.7 ± 7.3	0.99	**	***	***
	F <sub>3</sub> J	87.1 ± 8.9	0.77	0.77	–	–	–	–	–
	F <sub>3</sub> MS	86.8 ± 9.0	0.50	0.34	–	–	–	ns	–
Stem length (Sl, cm)	F <sub>2</sub> J	72.6 ± 6.9	0.72	–	68.3 ± 6.1	0.93	****		
	F <sub>2</sub> MS	66.3 ± 7.1	0.74	–	72.7 ± 5.6	0.96	****	***	***
	F <sub>3</sub> J	69.6 ± 7.4	0.68	0.65	–	–	–	–	–
	F <sub>3</sub> MS	68.5 ± 7.6	0.39	0.28	–	–	–	ns	–
Internode length (IL, cm)	F <sub>3</sub> J	12.9 ± 2.4	0.14	0.14	–	–	–	–	–
	F <sub>3</sub> MS	11.5 ± 2.4	0.11	0.00	–	–	–	***	–
Panicle length (PL, cm)	F <sub>2</sub> J	16.9 ± 2.1	0.49	–	16.5 ± 2.5	0.75	ns		
	F <sub>2</sub> MS	18.2 ± 2.9	0.67	–	17.7 ± 2.9	0.71	ns	***	**
	F <sub>3</sub> J	17.4 ± 2.6	0.87	0.87	–	–	–	–	–
	F <sub>3</sub> MS	18.3 ± 3.0	0.86	0.86	–	–	–	***	–
Flag leaf length (FL, cm)	F <sub>2</sub> J	20.8 ± 4.1	0.00	–	22.3 ± 5.0	0.55	**		
	F <sub>2</sub> MS	22.5 ± 5.0	0.39	–	24.0 ± 4.8	0.50	*	***	*
Culm diameter <sup>[2]</sup> (CD, mm)	F <sub>2</sub> J	5.05 ± 0.98	0.34	–	3.95 ± 0.87	0.24	****		
	F <sub>2</sub> MS	5.10 ± 0.94	0.35	–	3.87 ± 0.89	0.00	****	ns	ns
	F <sub>3</sub> J	7.09 ± 2.27	0.62	0.07	–	–	–	–	–
	F <sub>3</sub> MS	7.46 ± 2.20	0.00	0.00	–	–	–	***	–
Panicle number <sup>[2]</sup> (PN)	F <sub>2</sub> J	17.8 ± 1.4	0.00	–	5.4 ± 1.4	0.00	****		
	F <sub>2</sub> MS	12.0 ± 1.3	0.00	–	5.3 ± 1.3	0.39	****	***	ns
	F <sub>3</sub> J	11.8 ± 1.4	0.05	0.00	–	–	–	–	–
	F <sub>3</sub> MS	11.8 ± 1.4	0.21	0.00	–	–	–	ns	–
Total panicle weight (TW, g)	F <sub>2</sub> J	80.1 ± 22.1	0.40	–	20.1 ± 6.74	0.00	****		
	F <sub>2</sub> MS	51.0 ± 11.8	0.00	–	18.4 ± 5.77	0.00	****	***	ns
	F <sub>3</sub> J	53.9 ± 17.6	0.00	0.00	–	–	–	–	–
	F <sub>3</sub> MS	53.7 ± 19.5	0.00	0.00	–	–	–	ns	–
Mean panicle weight (PW, g)	F <sub>2</sub> J	4.39 ± 0.66	0.91	–	3.63 ± 0.61	0.64	****		
	F <sub>2</sub> MS	4.19 ± 0.77	0.94	–	3.41 ± 0.74	0.78	****	ns	*
	F <sub>3</sub> J	4.43 ± 0.75	0.49	0.43	–	–	–	–	–
	F <sub>3</sub> MS	4.37 ± 0.83	0.48	0.00	–	–	–	ns	–
Days to 95% maturity (MAT)	F <sub>2</sub> J	135.7 ± 4.9	1.00	–	136.6 ± 5.7	1.00	ns		
	F <sub>2</sub> MS	134.3 ± 3.9	1.00	–	131.3 ± 5.4	1.00	****	ns	****
	F <sub>3</sub> J	138.8 ± 5.9	0.97	–	–	–	–	–	–
	F <sub>3</sub> M	132.8 ± 4.6	0.97	–	–	–	–	***	–
Growth habit (GH)	F <sub>2</sub> J	3.20 ± 0.64	1.00	–	4.36 ± 0.65	0.61	****		
	F <sub>2</sub> MS	3.42 ± 0.72	1.00	–	4.30 ± 0.85	0.63	****	ns	ns
	F <sub>3</sub> J	3.60 ± 0.80	0.08	–	–	–	–	–	–
	F <sub>3</sub> MS	3.31 ± 0.78	0.46	–	–	–	–	**	–
Pulling resistance <sup>[2]</sup> (PR)	F <sub>3</sub> J	3.63 ± 1.38	0.68	0.00	–	–	–	–	–
	F <sub>3</sub> MS	3.12 ± 1.41	0.65	0.00	–	–	–	***	–
100–grain weight (g)	F <sub>2</sub> J	3.63 ± 0.29	0.59	–	3.59 ± 0.38	0.89	ns		
	F <sub>2</sub> MS	3.83 ± 0.26	0.55	–	3.51 ± 0.41	0.91	****	***	ns
Mean grain length (GL, mm)	F <sub>2</sub> J	6.25 ± 0.31	0.96	–	6.32 ± 0.32	0.97	ns		
	F <sub>2</sub> MS	6.56 ± 0.21	0.75	–	6.53 ± 0.28	0.94	ns	***	****
Mean grain width (GW, mm)	F <sub>2</sub> J	3.34 ± 0.09	0.77	–	3.36 ± 0.10	0.88	ns		
	F <sub>2</sub> MS	3.35 ± 0.14	0.72	–	3.30 ± 0.11	0.62	ns	ns	**
GL/GW (GS)	F <sub>2</sub> J	1.88 ± 0.10	0.93	–	1.88 ± 0.09	0.90	ns		
	F <sub>2</sub> MS	1.97 ± 0.10	0.94	–	1.98 ± 0.09	0.88	ns	***	***
% white–cored grains <sup>[2]</sup> (WP)	F <sub>2</sub> J	67.3 ± 10.9	0.69	–	76.3 ± 7.9	0.36	ns		
	F <sub>2</sub> MS	87.5 ± 7.60	0.53	–	83.2 ± 7.3	0.90	ns	***	ns
Mean white core size (WS)	F <sub>2</sub> J	1.78 ± 0.46	0.43	–	2.21 ± 0.37	0.91	****		
	F <sub>2</sub> MS	1.93 ± 0.40	0.56	–	1.75 ± 0.41	0.93	**	*	****

<sup>[1]</sup>ANOVA contrasts: E, Field vs basins; Pf, Pb, J vs MS in field or in basins, respectively. ns, \*, \*\*, \*\*\*\*, not significant, significant at  $p(\alpha) \leq 0.05$ ,  $p(\alpha) \leq 0.01$  and  $p(\alpha) \leq 0.001$ , respectively. Shadowed significances indicate significant differences also when checks were compared. Underlined significances indicate that the differences are significantly bigger than between checks. <sup>[2]</sup>H<sup>2</sup> calculated with transformed data.

**Table 3.** Factor pattern and eigenvalues of principal components of traits measured in F<sub>2</sub>.

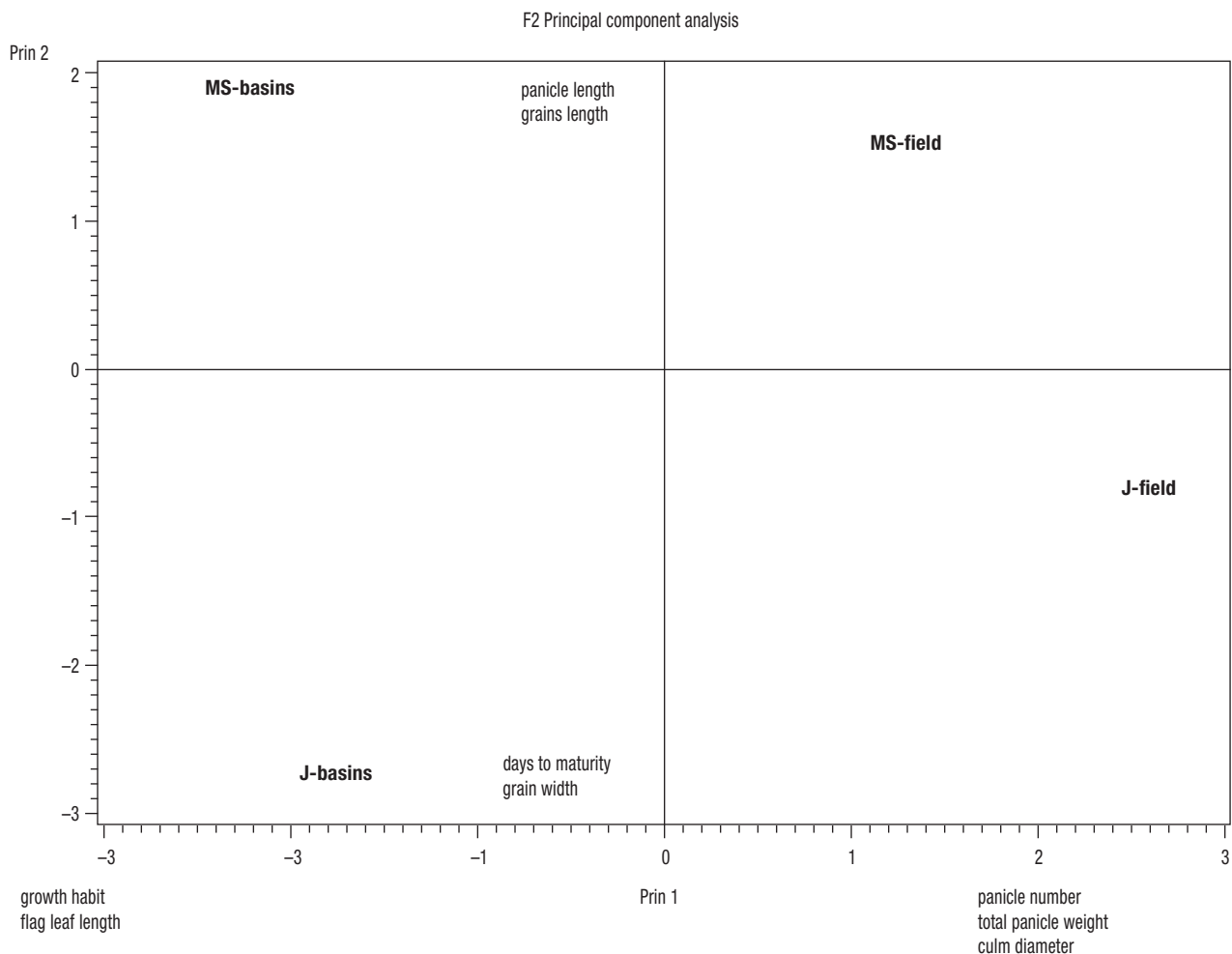
Traits	Principal components		
	1	2	3
Plant height	-0.03	0.17	-0.56
Mean panicle length	-0.03	0.45	0.11
Mean flag leaf length	-0.35	0.26	-0.02
Mean culm diameter	0.41	0.07	0.14
Log (panicle number)	0.42	0.04	-0.05
Total panicle weight	0.42	-0.004	-0.13
100-grain weight	0.25	0.16	0.44
Mean grain length	-0.02	0.40	0.29
Mean grain width	-0.20	-0.38	0.18
$\text{Arcsin } \sqrt{\%(\text{chalky grains})}$	-0.17	0.31	0.38
Mean grain chalk size	-0.14	-0.33	0.37
Days to 95% maturity	0.18	-0.39	0.18
Growth habit	-0.42	-0.08	-0.02
Eigenvalue (variance explained)	5.6	4.8	2.7
% of variance explained	42.8	36.8	20.5

individuals selected in basins or field. Table 4 shows the population means of traits evaluated in selected F<sub>2</sub> and F<sub>3</sub> plants within the breeding programme (except maturity, which was not accurately estimated in the F<sub>2</sub>). The last column is a rough comparison between environments. Number of panicles was the only trait which presented significant differences between F<sub>3</sub> plants selected from different F<sub>2</sub> environments: Those derived from field selection tillered more than those derived from basins' selection. The selection of the other traits was not influenced either by the environment or the different selection pressure used.

### Lodging selection

In both crosses, F<sub>3</sub> selection in the field ("lodging selection") was effective in reducing plant height and in increasing culm diameter and internode length (Table 5).

In F<sub>4</sub>J, panicle number and pulling resistance were also effectively increased. However, internode length

**Figure 2.** Plot of the two first principal components on four F<sub>2</sub> population/environment combinations.

**Table 4.** Populations means of traits evaluated in globally selected F<sub>2</sub> and F<sub>3</sub> plants

Character	Cross	F <sub>2</sub> field	F <sub>2</sub> basins	F <sub>3</sub> from F <sub>2</sub> field	F <sub>3</sub> from F <sub>2</sub> basins	Significance of the difference of F <sub>3</sub> means <sup>[1]</sup>
Plant height	J	81.9	73.7	–	76.3	–
	MS	79.0	75.2	80.3	80.7	t <sub>19</sub> =0.17 ns
Panicle length	J	18.5	18.5	–	20.2	–
	MS	21.7	18.8	18.0	19.3	t <sub>19</sub> =1.14 ns
Nº of panicles	J	18.6	8.2	–	12.5	–
	MS	13.3	6.2	24.8	22.1	t <sub>19</sub> =2.63 *
Total panicle weight	J	88.2	30.4	–	91.7	–
	MS	66.1	26.8	121.8	113.1	t <sub>19</sub> =0.68 ns

<sup>[1]</sup>t<sub>19</sub>: result of the Student t test with 19 degrees of freedom. ns, not significant. \*, significant at  $p(\alpha) \leq 0.05$ .

**Table 5.** Response to F<sub>3</sub> selection in the field, in lodging related traits.

Cross	Character	Overall F <sub>3</sub> mean	Selected F <sub>3</sub> mean	F4 mean	S <sup>[1]</sup>	R <sup>[2]</sup>
J	Plant height (–)	87.06	72.52	63.29	–14.54	–23.77 ***
	Culm diameter (+)	0.72	0.84	0.97	0.12	0.25 ***
	Panicle number (+)	12.32	16.58	18.19	4.26	5.87 ***
	Panicle number (–)	12.32	8.86	13.08	–3.46	0.76 ns
	Internode length (+)	12.94	16.08	15.85	3.14	2.91 ***
	Internode length (–)	12.94	10.19	12.86	–2.75	–0.08 ns
	Pulling resistance (+)	3.63	4.74	4.73	1.11	1.10 *
MS	Plant height (–)	86.76	74.58	67.29	–12.18	–19.47 ***
	Culm diameter (+)	0.75	0.92	0.97	0.17	0.22 ***
	Panicle number (+)	12.47	18.85	15.12	6.38	2.65 ns
	Panicle number (–)	12.47	8.56	11.86	–3.91	–0.61 ns
	Internode length (+)	11.53	15.52	15.87	3.99	4.34 ***
	Internode length (–)	11.53	8.61	12.33	–2.92	0.80 ns
	Pulling resistance (+)	3.12	4.39	3.59	1.27	0.47 ns

<sup>[1]</sup>S: selection differential. <sup>[2]</sup>R: Response to selection. ns, \*, \*\*\*: no significant, significant at  $p(\alpha) \leq 0.05$  and  $p(\alpha) \leq 0.005$ , respectively.

and number of panicles did not show a significant response to negative selection.

## Discussion

### Preliminary results

Comparing the success of both types of selection in the F<sub>2</sub> of the nine varieties obtained in the last years, narrow-spaced selection seems quantitatively more effective than wide-spaced selection, however 'JSendra' (selected in the field, widely-spaced) is the most cultivated variety among the nine.

### Experimental results

As expected, most of the traits showed higher expression (growth potential) in the field under wide spacing

(except FL in both populations, and PH in F<sub>2</sub>MS). The traits which showed higher heritability (H<sup>2</sup>) in the field were PW, GH, CD, GW and WP (in both populations), and TW in F<sub>2</sub>J. In basins, the shadow avoidance response and the stronger competition under a higher planting density restricted plant yield (panicle number and weight, and consequently total weight) and stem thickness; plants also grew more open in basins. But the weaker environmental influence in basins than in field plots resulted in higher heritabilities for most traits (ten). This can be explained by the fact that basins are a more homogeneous environment, demonstrated by the lack of significant differences found when comparing checks (shadowed entries of the last column in Table 2).

This study detected genetic, environmental and G×E effects in some of the traits studied. Most of the genetic and environmental differences were confirmed by principal components analysis, whose first axis is correlated with traits with significant and consistent differences between basins and field (PN, TW, CD, GH and FL),



and therefore differentiates field values from basin values. Tillering (PN), and consequently yield, is well known for being highly sensitive to environmental factors, including cultural practices such as planting density (Martínez, 2010). Broad sense heritabilities of these traits were low, in agreement with the strong environmental influence, and with previous studies (see Yamagata, 1997 for FL, or Xiong, 1992 for PN). An exception was growth habit in the  $F_2$ , but its  $H^2$  turns to moderate-low in  $F_3$ . The results concerning the  $F_2$  populations are, however, approximate, since each combination population/environment consisted of 75 plants only.

The second component reflects the influence of genotype on traits such as earliness, panicle structure and grain shape (MAT, PL, GL and GW). Although most of the traits showed some significant difference between crosses in  $F_2$  or  $F_3$ , the four traits mentioned above are the only ones where such differences were consistent in both environments and generations. Broad sense heritabilities of these traits were, accordingly, quite high in  $F_2$ . In fact, grain dimensions are easily fixed in early generations, and MAT is so from the  $F_3$  (Jennings *et al.*, 1979).

Some characters (PH, SL, and WS) displayed  $G \times E$  interaction. This was due to the particular situation of the field used for the study of  $F_2MS$ : It was placed along a natural barrier which caused the rise of temperature and humidity levels (Fig. 1b,c). An environmental difference between both  $F_2$  fields was therefore probably the cause of the apparent  $G \times E$  interaction, and also the cause of the generally higher difference in trait means between the  $F_2$  populations in the field than between the  $F_3$  populations (except for CD and GH).

We can see true  $G \times E$  interactions when comparing the behaviour of the checks vs that of the  $F_2$  or  $F_3$  populations: Although most significant differences between field plots and basins for both  $F_2$  populations are maintained when comparing the checks, both  $F_2J$  and  $F_2MS$  had thicker culms and shorter flag leaves in the field than in the basins, while checks did not differ when comparing both environments. And on the contrary, the environment had a stronger influence on PL of 'Senia' checks than on the same trait in  $F_2J$  or  $F_2MS$ . Another trait which shows environmental plasticity in 'Senia' is the presence and length of awns (data not shown).

In traits such as PH (and SL), MAT and grain traits (weight, dimensions and white core presence),  $R^2$  values (coefficients of determination) are very low, both in genotypes' and environments' ANOVAs (data not shown) of the  $F_2$ , indicating that  $G \times E$ , not included in the model, must be an important factor in determining variation. On the other hand, in yield related traits (PW, PN, TW), as well as GW and CD, the ANOVA across

environments explains better their variation than the ANOVA across genotypes. Finally, PL and FL are traits whose variation is mainly determined by both "population" and "environment" factors.

As mentioned earlier, populations  $F_3J$  and  $F_3MS$  only differed significantly in a few traits. These differences can be assigned to genetic differences and the  $G \times E$  component (underlined significances in Table 2); this is in agreement with the higher  $R^2$  obtained in the  $F_3$  ANOVA, where the model (which leaves the environmental variance in the residue) explains quite well the observed variation. This is because their field plots were adjacent and highly comparable, as demonstrated by the checks' contrasts of significance (Table 2).

## Global selection

Although we have noted before that the difference in selection pressure did not affect the majority of traits evaluated, we may draw attention that in the field it doubled that in basins. The reasons for the higher selection pressure applied on the  $F_2$  selected in the field are two: 1) within our breeding program, we devoted at least two weeks to select four or five  $F_2$ s in basins, while field selection required only one day per  $F_2$ , which can affect the number of plants that have reached maturity that given day; 2) wide-spaced plants are easier to differentiate, because in the field plants were more productive and tillered more. Therefore, they can be selected more strictly.

Response to selection could not be determined for two reasons: because the only plants measured were the selected plants (not the whole  $F_2$  and  $F_3$  populations) and because generations were grown in different years and conditions.

From the results of the MS cross, of four characters, only panicle number showed significant differences between environments of origin in the  $F_2$ : the  $F_3$  derived from field  $F_2$  continued to tiller more than those derived from  $F_2$  selected in basins, under the same  $F_3$  plant density. This can be explained in this way: since plants tiller more under wide spacing, higher tillering genotypes were more effectively selected in the field, and some genetic differences in panicle number have been fixed; although they were not detected in the heritabilities calculated by ANOVA, probably because they are less reliable estimators than realized heritability ( $h^2=R/S$ ). Plant yield had the same tendency, but differences were not significant. It may be concluded that both environments (field and basins) can be used for selection in the  $F_2$ , although some different response might be expected in yield related traits; but we cannot draw conclusions out of only three lines selected in the field.

## Lodging selection

The objectives were: (1) to see the effect of a moderate reduction in height as tall plants are clearly prone to lodging, but not drastic enough to compromise yield; 2) to analyse the effect of reducing the length of the first internode on total plant height; 3) to determine if longer internodes can contribute to the resistance of the basal part of the rice plant (by improving its elasticity, and so its ability to recover), that was the reason for applying both positive and negative selection to IL to increase stem sturdiness by selecting thicker culms; 4) to see the effect of a change in panicle number; and 5) to check if the measure of the pulling resistance can serve as an estimator of susceptibility to lodging. It was not surprising that selection succeeded in reducing plant height, as this trait showed a high heritability. But the effective increase in stems diameter or internode length was unexpected, considering the observed low heritabilities, with no significant additive variance in one of the crosses. One possible explanation for the improvement of culm diameter lies in the presence of several QTLs displaying overdominance in the selected MS lines (QTL analysis was not performed in the J cross; Torr , 2010). As for IL, interestingly selection for obtaining plants with shorter internodes was useless in both crosses. This suggests that the observed reduction in plant height in both crosses after selection was achieved either by shortening of other internodes, or by reducing the number of nodes (which is different among the ancestors of both populations); but these traits were not evaluated in the segregating populations. The other two traits with low heritabilities that responded to selection —but only in the J cross—, were panicle number and pulling resistance. Similarly to IL, only positive selection to increase PN was effective; again, this might be due to the abundant positive dominance and overdominance observed in the QTLs for PN (Torr , 2010). Alternatively, the response to selection in traits with low observed heritability could be due, again, to the existence of additive variance not detected through analysis of variance of sibs, since realized heritability is more reliable. Finally, pulling strength showed correlation with lodging susceptibility (Torr  *et al.*, 2011), but in that study we concluded that the angle difference of recovery after bending a stem would be a more advisable criterion for selection of lodging resistance.

We may conclude that the two ways of F<sub>2</sub> cultivation described can be used for selection, although different responses might be expected in yield related traits.

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