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Crossability analysis of cultivated rice (*Oryza sativa*) with *O. rufipogon* and *O. longistaminata* and F_1-identification

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Perennial rice (*Oryza sativa* L.) is one that lives and remains productive for two or more years, rather than growing only for one season till harvest. Some wild species of rice like *O. longistaminata, O. rhizomatis* and *O. rufipogon* show perennial characteristics as these species have plant structures like rhizomes and stolons and thus, have the capacity to regenerate making the plant perennial. Perennation in rice is often manifested as the ratooning ability (re-growth) of the plant. The perennial nature and ratooning ability of rice is linked to their evolutionary pathway. Cheng *et al.* (2003) suggested that *O. rufipogon* which was the progenitor species of *O. sativa*, consisted of four major clades, three of which were perennial and one was annual (the annual clade is referred to as *O. nivara*). He postulated that the *indica* sub-species of *O sativa* was derived from the annual *O. rufipogon* clade while the *Japonica* subspecies was derived from one of the perennial *O. rufipogon* clades. Thus, many rice cultivars grown today were originally perennials that are now cultivated as annuals.

Perennation in *O. sativa* is by growth of axillary buds on older tillers (i.e. tillering), whereas *O. rufipogon* can additionally propagate from stolons, and *O. longistaminata* produces many long rhizomes that are the primary source of new shoots. *O. rufipogon* has high cross compatibility with *O.sativa*. However, stolon is not well suited for surviving in drought since, due to exposure in sun, the stolon become dry and unproductive. In contrast, *O. longistaminata* produces rhizomes which are protected from desiccation by insulating soil. Breeding barriers however have severely limited the production of *O. sativa*/*O. longistaminata* F_1 progeny. Genotypes of *O. sativa* vary greatly in their propensity to ratoon after an initial harvest of grains (Prakash *et al.* 1988, Chauhan *et al.* 1989). Hence, the variability in ratooning ability could be exploited for development of good ratooning or perennial rice varieties.

Present study was carried out at the Instructional cum Research (ICR) farm in Assam Agricultural University, Jorhat, Assam for the year 2019–21 with the objective to determine the crossability of wild rice species *O. longistaminata* and *O. rufipogon* with *O. sativa* and observe the inheritance of rhizome. Five popular cultivars of rice, viz. Ranjit, Ranjit sub-1, Binadhan-11, IR-64 and Bahadur were chosen (from first year of experiment) as these varieties also possess some specific valuable distinguished traits like good ratooning ability (Chakrawarti *et al.* 2022). These selected varieties were crossed (second year) as female parent with *O. longistaminata* and *O. rufipogon* as male parent. Reciprocal crossing was also done. Crossability was determined by counting the number of seeds produced per cross. It was calculated as the ratio of number of true F_1 seeds developed per cross to the total number of spiklet emasculated and was expressed as percentage.

$$\text{Crossability} = \frac{\text{Total } F_1 \text{ seeds produced}}{\text{Total number of spikelet emasulated}} \times 100$$

For pollen viability test, five spikelets from five random plants in parent and single plant in F_1 were collected at the stage before flower opening in the test tube containing 70% ethyl alcohol. Anthers were then squashed with 1% Iodine Potassium iodide (IKI) solution and examined under light microscope. The pollen viability was calculated as:

$$\text{Pollen viability} = \frac{\text{No. of viable pollen grains}}{\text{Total no. of pollen grain}} \times 100$$

Crossability was estimated in two ways. Crosses were made between *O.sativa* as female and wild rice as male. Reciprocal crosses were also made. The number of true F_1s after the hybridization between *O. sativa* varieties as female and *O. longistaminata* as male were found varying according to the varieties used as female. The highest percentage of seed set was found in the cross

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Binadhan-11 × *O. longistaminata* with the crossability of 22.2% followed by the cross IR-64 × *O. longistaminata* (19.4%), Ranjit × *O. longistaminata* (16.3%) and Bahadur × *O. longistaminata* (11.8%). Lowest seed set was found in the cross Ranjit Sub-1 × *O. longistaminata* (11.1%).

The number of F_1s after the hybridization between *O. sativa* varieties as female and *O. rufipogon* as male was found different with different female parents. The number of fertile spikelets were very less (Abraham *et al.* 2021) . The highest percentage of seed set among *O. sativa/O. rufipogon* was found in the cross Ranjit × *O. rufipogon* (30%) followed by the cross Binadhan-11 × *O. rufipogon* (26.2%), IR64 × *O. rufipogon* (22.2%), Bahadur × *O. rufipogon* (19.4%), whereas lowest crossability was exhibited by the cross Ranjit sub-1 × *O.rufipogon* (13.3%). The comparable result for crossability between *O. sativa/O. longistaminata* was also obtained by Dayun and Sripichitt (2000). They also suggested that the extent of crossability between *O. sativa* and wild rice is mainly decided by pollen fertility of the male parent and the environmental factors. For *O. sativa/O. rufipogon*, similar and comparable crossability percentage was obtained by Niruntrayakul *et al.* (2009). The F_1 from *O. sativa/O. longistaminata* were further observed and could be differentiated from parents in terms of several qualitative and quantitative traits. It has also been suggested that this diversity in the reproductive isolation is mainly due to diversity in flowering time (Xu *et al.* 2020). Hybrids were found intermediate between the two parents for many distinguishing traits. None of the F_1 seeds could be recovered from the crosses in which *O. longistaminata* and *O. rufipogon* was used as female parent.

Further evaluation was done for F_1s from *O. sativa/O. longistaminata* for various contrasting morphologically expressed qualitative (Table 1) and quantitative traits (Table 2) of vegetative and reproductive stage till the duration of investigation because hybrid from IR64 × *O. longistaminata* have not come to reproductive stage. Hence, for this cross none of the traits expressed at reproductive stage were observed. It is worth to note that IR-64 is photoinsensitive in nature, however, its hybrid with *O. longistaminata* were photosensitive thus, they could not come to reproductive stage till the time of research (August 2021).

F_1s were poor performing in terms of pollen fertility, spikelet fertility and number of filled grains. The F_1s had lower pollen viability as compared to both the parents in all the crosses. Low level of pollen fertility was also observed by Lu *et al.* (2003) while Sacks *et al.* (2006) observed spikelet fertility as low as 13% in F_1 from *O. sativa/ O. longistaminata*. Low number of seed set in such hybrids was probably due to meiotic irregularities as reported by Kaushal *et al.* (1998). Li *et al.* (2007) concluded that the sterility of F_1 was the main barrier in gene flow within AA genome. Apart from this, positive heterosis for flag leaf length was observed in hybrid from cross Ranjit sub-1 × *O. longistaminata,* Bahadur × *O. longistaminata* and IR64 × *O. longistaminata* and longest flag leaf length was recorded in hybrid from IR64 × *O. Longistaminata*

Table 1 Comparision of various qualitative traits among parents and hybrids

	Stem base colour	Leaf blade pubescence	Basal leaf sheath colour	Flag leaf angle	Internode colour	Panicle type	Awns	Anther colour	Stigma colour	Stigma exertion
Male parent										
O. longistaminata	Purple	Pubescent	Light purple	Erect	Light purple	Open	Long and fully awned	Pale yellow	Purple	Fully exerted
Female parent										
Binadhan-11	Green	Glabrous	Green	Erect	Green	Compact	Awnless	Yellow	White	No exertion
IR-64	Green	Glabrous	Green	Slightly drupy	Green	Compact	Awnless	Yellow	White	No exertion
Ranjit	Green	Glabrous	Green	Slightly drupy	Green	Compact	Awnless	Yellow	White	No exertion
Ranjit sub-1	Green	Glabrous	Green	Slightly drupy	Green	Compact	Awnless	Yellow	White	No exertion
Bahadur	Green	Glabrous	Green	Erect	Green	Compact	Awnless	Yellow	White	No exertion
F_1										
Binadhan11 × *O. longistaminata*	Purple	Glabrous	Green	Erect	Green	Intermediate	Awn only on top grain	Pale yellow	Purple	Partially exerted
Ranjit sub-1 × *O. longistaminata*	Green	Glabrous	Green	Erect	Green	Intermediate	Long and fully awned	Light yellow	White	Partially exerted
Bahadur × *O. lonistaminata*	Green	Glabrous	Green	Erect	Green	Open	Long and fully awned	Light yellow	White	Partially exerted
Ranjit × O. longistaminata	Green	Glabrous	Green	Erect	Green	Intermediate	Partially awned	Light yellow	Purple	Partially exerted
IR64 × *O. longistaminata*	Green	Glabrous	Green	Erect	Green	-	-	-	-	-

Table 2 Comparision of hybrid with their parents for quantitative characters

	No of tillers	Flag leaf length (cm)	Flag leaf width (cm)	Panicle length (cm)	No.of fertile grain per panicle	Pollen viability (%)
Male parent						
O. longistaminata	7	38.5	1.00	39.5	47	86.5
Female parent						
Binadhan-11	12	33.2	1.3	24.2	103	100
IR-64	16	53.2	1.0	22.3	83	100
Ranjit	10	22.4	1.6	29.4	120	100
Ranjit sub-1	12	45.2	1.2	27.3	135	100
Bahadur	11	60	1.5	30.5	102	100
F_1						
Binadhan11 × *O. longistaminata*	21	29.2	1.1	22.5	5	32.5
Ranjit sub-1 × *O. longistaminata*	30	59.2	1.1	30.2	10	36.4
Bahadur × *O. longistaminata*	35	71.5	0.7	34.5	5	30
Ranjit × *O. longistaminata*	26	24.7	1.00	19.6	7	40.2
IR64 × *O. longistaminata*	23	86.5	1.5	-	-	-

(86.5 cm). Width of flag leaf were intermediate for all the hybrids. Negative heterosis for panicle size was seen in hybrids from Binadhan-11 × *O. longistaminata* (22.5 cm) and Ranjit × *O. longistaminata* (19.6 cm). All the F_1 hybrids from *O. sativa*/*O. longistaminata* possessed awns as that of the male parent. However, the intensity of awns was found varying with the cross. Hybrid from Ranjit sub-1 × *O. longistaminata* and Bahadur × *O. longistaminata* had long awned spikelets. The F_1 from Binadhan-11 × *O. longistaminata* had purple stem as that of male parent. This character can be preferably used as morphological marker at vegetative stage to assist hybrid selection in early stages. However, number of fertile grains per panicle were found very less in all the hybrids, highest being 10 seeds hybrid from Ranjit sub-1 × *O. longistaminata*. Pollen viability of all the hybrids were less than both the parents and least pollen viability was observed in hybrid from Bahadur × *O. longistaminata* (30%)

SUMMARY

The research was conducted at Instructional cum Research (ICR) farm in Assam Agricultural University, Jorhat, Assam for the year 2019–21 with the objective to determine the crossability of wild rice species *O. longistaminata* and *O. rufipogon* with *O. sativa* and observe the inheritance of rhizome. In first year of experiment, varieties having good ratooning ability were identified. Out of them, five varieties namely Ranjit, Ranjit sub-1, IR-64, Binadhan-11 and Bahadur were crossed with both of the wild species. Crossablity of *O. longistaminata* with *O. sativa* ranged from 11.1% (Ranjit sub-1 × *O. longistaminata)* to 22.2% (Binadhan11 × *O. longistaminata)* while *O. rufipogon* with *O. sativa* ranged from 13.3% (Ranjit sub-1 × *O. rufipogon)* to 30% (Ranjit × *O. rufipogon)*. All the hybrids except for IR64 × *O. longistaminata* were photoinsensitive as that of their female parent. Hybrids from *O. sativa/O. longistaminata* exhibited various distinguishing characters which can be used as morphological marker in order to identify true interspecific hybrids in further breeding programme. The pollen viability of these interspecific hybrids were found lower than both the parents. However, rhizome formation could not be observed in any hybrid.

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