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Short Communication

Speed breeding: to expedite research and breeding in Rice

Kaldate Supriya, Pandit Praveen and Daisy Basandrai*
Department of Genetics and Plant Breeding, College of Agriculture
CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176 062, India.

*Corresponding author: daisybasandrai@gmail.com
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Abstract

To meet the growing population's food requirements, speed breeding could be the most appropriate strategy to develop improved cultivars with high yield potential, and it can enhance research in agriculture by reducing the generation time. However, the strategy involves a costly setup and is quite expensive. In hilly and temperate regions of India, only one crop can be taken in the monsoon season, as the temperature drops in October, it restricts breeding advancement. In rice, with the manipulation of photoperiod requirements in controlled conditions inside a polyhouse, three to four crops can be taken within a single year, which will speed up cultivar development.

Key words: Speed breeding, Rice, Photoperiod

To feed the growing population, plant breeders are continuously engaged in improving the genetic makeup of crop plants to attain higher crop yields. The global impact of climate change on food security for the coming generations also cannot be neglected. To tackle the growing food demands in this changing environment, we need a technique that can keep pace with time. Traditional plant breeding methods for cultivar development take a lot of time and require many generations of evaluation before the release of cultivars. Plant breeders are continuously engaged in developing techniques to hasten the generations, such as shuttle breeding, doubled haploidy breeding (DH), marker-assisted selections (MAS), genomic selection, etc. The technique that uses artificial light and temperature conditions to speed up crossing and inbreeding has been developed and is known as speed breeding. Among all these techniques, speed breeding serves as a flexible tool to shorten the breeding cycle by manipulating photoperiod to accelerate the development rate of plants and generation time. Speed breeding was successfully deployed in bread wheat (*Triticum aestivum* L.) to achieve six generations per year while imposing phenotypic selection for foliar disease resistance and grain dormancy (Alahmad *et al*. 2018). Speed breeding has been suggested to speed up selection in early generations as it provides increased recombination during line development over DH (Double Haploid). Breeding approaches that manipulate the circadian clock have great potential to shorten the reproductive period in the case of long-day plants. This technique was applied to rapidly introgress rust resistance into several Australian cereal cultivars (Hickey *et al*. 2015). With the extended photoperiod utilising supplementary light and temperature control with sodium vapour lamps (SVL) or growth chambers fitted with a mixture of metal halide and light-emitting diode (LED) lighting, generation advancement can be done in glasshouses. Speed breeding can be used to achieve up to 6 generations per year for spring wheat (*Triticum aestivum*), durum wheat (*T. durum*), barley (*Hordeum vulgare*), chickpea (*Cicer arietinum*), and pea (*Pisum sativum*), and 4 generations for canola (*Brassica napus*) under normal glasshouse conditions (Watson *et al*. 2018). They also evaluated the effect of the loss of function of *FLOWERING LOCUS T-B1* in the recombinant inbred line of Paragon x W352 and observed booting and spikelet development at 44 and 67 days, respectively. For improving the stay-green and root adaptation of wheat in changing water-limited Australian environments, speed breeding was employed to take 7 generations per annum, and the NAM (nested association mapping) population was accelerated up to 5 generations within 18 months (Christopher *et al*. 2015). Speed breeding combined with genomic selection (GS) allows rapid and higher genetic gains in allogamous crops (Jighly *et al*. 2019). Rice is a short-duration crop and can be grown only in *kharif* (June–October), when the climate is hot and humid. In northern Indian plains and hilly areas, lower temperatures limit the advancement of rice breeding programmes. With a minimal setup of polyhouses, four generations can be taken to speed up the breeding cycle.

Circadian Clock and Speed Breeding

For maintaining a precise reproductive cycle short and long-day plants have different photoperiod requirements. Circadian clocks are endogenous time-keeping mechanisms that allow organisms to anticipate and prepare for daily and seasonal changes in surrounding environments. The circadian clock controlling genes measure the day length based on the appropriate season and time of the day and accordingly adjust various physiological, developmental and reproductive processes (McClung 2006). This mechanism in plants is embarked by environmental factors like light, temperature and nutrient status (Inoue *et al*. 2017). It has been suggested that modifying circadian rhythms may be a means to manipulate crops to develop improved plants for agriculture. Extended photoperiods under controlled conditions can overdrive photosynthesis resulting in faster growth and reduced generation time in many crops. This is achieved by the use of LED lights indoors.

Two species of *Oryza* viz., *sativa* and *glaberrima* were used. Details of the material used in this study are given in Table 1. Seedlings were raised by the top of the paper method using germination paper. Seeds were placed on moistened filter paper in Petri plates and placed in an incubator with T 27±2^{0}C and RH 92%. After 6-7 days when sprouts reached 5-7cm height and 2 leaves stage, such plants were transplanted into pre-prepared pots inside the polyhouse.

Table 1. Genotypes used in this study

Sr. No.	Genotype	Parentage/Source
1	HPR-1068	IR 42015-83-2-2/IR 9758-K2
2	HPR-2795	Pure line selection from Sakura red
3	*O. glaberrima*	Collection from Nigeria

The off-season rice trial was sown in the first week of December 2019 in the automated polyhouse of the Department of Genetics and Plant Breeding, CSK HP Krishi Vishvavidyalaya, Palampur. To compare the flowering time between field and polyhouse conditions, data were recorded on plot basis in three replications in field. In polyhouse data was recorded for five plants with three replications. Rice is the major food of the Asian continent.It is the short day plant with optimum temperature of 23^{0}C for flowering and growth.Therefore, it can be cultivated in the period of June to September in India.In foothills of the Himachal, *Rabi* season starts from the mid October to the end of March. During this period normal temperature lies between 4-16^{0}C which is not suitable for paddy cultivation. Accelerating the life cycle aids in evading crops in temperate locations to prevent terminal chilling stress. Early maturity in case of pot culture in polyhouse was observed due to manipulation of photoperiod and temperature in the surrounding atmosphere. Barmudoi and Bharali (2016) observed increased growth of rice plant under normal white light as compare to low light regime.Winters in the Himachal region are bitterly cold, and sunlight hours are scarce. It's impossible to accelerate rice breeding in this region due to this climate regime unless a controlled environment is provided. The off season breeding work can be conveniently done under such an environment. In our off season breeding experiment, the temperature regime was kept at 20-25^{0}C and 15-20^{0}C for day and night, respectively, during the seedling growth phase using the four heaters fitted with an exhaust fan that helps to provide a uniform temperature. Using an artificial lighting system, 8–10 hours of light was applied for the normal growth of plants. During the reproductive phase, the temperature was kept at 25–32^{0}C and 20^{0}C, with humidity at 80–85% and 65-75% for the day and night, respectively. Flowering started in the third week of February. For the successful crossing, emasculation was done between 4-5 p.m., and pollination was done at 9-10 a.m. on the next day. Seed setting was observed after 10–12 days of pollination, with a seed-setting rate of 70–72 per cent. Additionally, it has been found that the use of humidifiers and moist gunny bags contributed to increased pollen production and pollen fertility. Elevated temperatures have been found to greatly affect pollen grains, producing morphological abnormalities, i.e., the shape and size of pollens (Kumar *et al.* 2019). This regime found to be the best for pre-breeding work under controlled conditions as compared to field experiments because, in rice season, the temperature often crosses 25^{0}C which leads to pollen sterility. During the maturity period, the humidity was maintained at 45–50%, which helped to promote early maturity. Both *O. glaberrima* and HPR 2795 are late maturing varieties. They took around 80 days to flower and 105–110 days to mature under polyhouse conditions. Under field conditions, both of them took 10–15 days longer to flower and reach maturity. The HPR 1068 is early maturing, it took 60 to 65 days to flower, 80 to 85 days to attain 75% maturity in a polyhouse, and 15-20 days more under field conditions. Mean values for the recorded traits are given in table 2. Paired t-test showed highly significant differences for days to 50% flowering and maturity duration between field and polyhouse condition. It was also observed that spikelet fertility was reduced in late maturing varieties (Figure 1). The early maturing varieties escape high temperature at the end of *kharif* season, which gives better fertility (Hu *et al*. 2021). The terminal chilling stress was found to be associated with heading difficulty and poor spikelet fertility (Ren *et al*. 2022). Apart from speed breeding with controlled conditions, many other methods have been suggested in rice to harvest 3-4 generations within a single calendar year viz., seed desiccation, direct seeding, shuttling for tropical environment etc. (Li *et al*. 2015). However, in northern foothills of Himalayas these methods are not feasible because of low temperature. Therefore for successful pre-breeding work, minimum setup of polyhouse with controlled condition is suggested.

Conclusion: In northern foothills of Himalayas rice breeding with minimum setup of polyhouse and mentioned conditions is suggested for advancing the generations. Compared to field conditions, polyhouse offers best conditions to perform pre breeding work

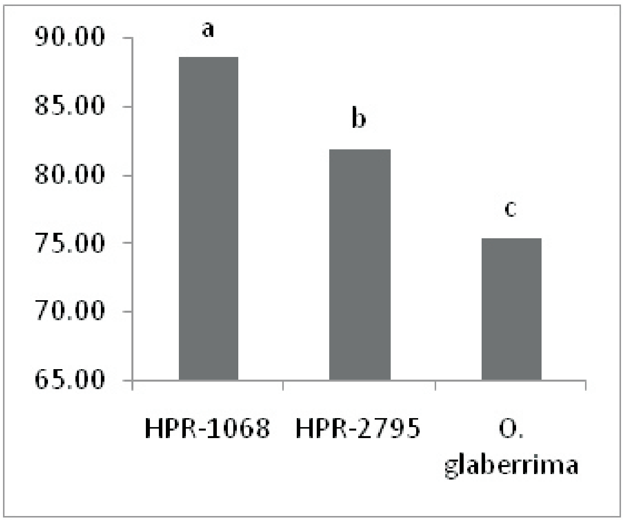

Figure 1. Spikelet fertility recorded under field conditions (Tukeys/HSD test at 5% level. Bars with different letter shows significant differences for spikelet fertility)

Table 2. Mean values for time of flowering in polyhouse and field conditions

Cultivar/spp.	*O. glaberrima*		HPR 2795		HPR 1068		T test
Trait	Polyhouse (Off- season)	Field (In season)	Polyhouse (Off -season)	Field (In season)	Polyhouse (Off- season)	Field (In season)	
Days to 50 %	79	94	81	90	59	79	0.0069*
flowering	80	92	82	92	62	77	
	80	95	80	91	56	75	
Days to maturity	105	114	110	104			
	109	112	104	120			0.0002*
	108	116	105	123			

*Significance at 1% (0.0069 and 0.0002 $\leq$ 0.01) and can advance 3-4 generations within a single calendar year.

Conflict of interest: The authors declare that there is no conflict of interest among the authors in this research paper.

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