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Harnessing Cooler Nights in the Central Zone of India to Achieve Speed Breeding Under Field Conditions

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ABSTRACT

The need to increase the annual genetic gain in crops continues to be urgent to sustain the growing food demand and the sustainability of the agri-food system. An important way to achieve this is by reducing the cycle time of crop breeding. There are various approaches where more than one generation of wheat can be grown each year, including by taking off-season crops. The greenhouse or artificial generation advancement facility is also used for quick generation advancement. However, it is costly and not suitable for developing countries or institutions having financial limitations. Moreover, the amount of breeding material to be handled in speed breeding facilities is also quite limited reducing the chance of obtaining all probable recombinant lines. Therefore, we explored a viable and cost-effective way to grow two wheat crops in a single season under natural field conditions in a location in the state of Madhya Pradesh, India where wheat has never been grown in the off-season. The experiment was conducted using six genotypes, varying in days to maturity on four different treatment combinations at the research farm of Borlaug Institute for South Asia (BISA), Jabalpur, Madhya Pradesh which falls under the Central Zone (CZ) of India. Out of four treatments, we got seed germination in two treatments with a success rate of >80%. The results proved that it is possible to obtain at least two generations of wheat crop under field conditions in the location used. This approach not only saves resources and time but also provides an opportunity to make selections in breeding populations at least from the first cycle. The results serve as a base to further refine this technique and eventually use it for wheat breeding or off-season multiplication of seeds to fast-track the entire process of varietal development and its dissemination.

Keywords: wheat, speed breeding, photoperiod, generation advancement, selection.

INTRODUCTION

In today's era, global food security is a significant concern. With the increasing population and climate change, it is most challenging to supply competing demands for food (Crist et al., 2017). Globally, wheat (*Triticum aestivum*) is the most consumed grain by humans among the cereals. Over the last ten years, globally, wheat consumption by humans has increased up to 90 million metric tons (MMT). Wheat is essential to the human diet, accounting for 20% of daily protein and calories. It is the second most important food crop in the developing world after rice in terms of food security, with an estimated 80 million farmers relying on it for their livelihoods (Crespo-Herrera et al., 2021).

Conventional varietal development and breeding programs worldwide have achieved remarkable growth in crop production and brought new varieties in the past century. Notwithstanding, such varietal development up to the release of variety had taken 10-15 years of the breeding cycle (Hickey et al., 2017).

Marker-assisted selection has been found helpful in the introgression of major genes in wheat in lesser time frame (Vishwakarma et al., 2014, 2016) but not so effective in introgression of a minor gene or quantitative trait loci (QTLs) (Cobb et al., 2019). In the recent past, Genomic selection (GS) has become popularized and considered an upgraded version of MAS because the selection of superior lines is done by applying genomic-estimated breeding values (GEBVs) based on high density genotyping; GEBVs calculation is done with the dataset of genotyping and phenotyping of the training

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population (Poland et al., 2012; Crossa et al., 2013). However, these tools take considerable number of years since to provide genetically static genotypes after rigorous selection. In this context, ‘speed breeding, has been found useful in reducing the length of the breeding cycle by taking several generations of wheat crop in a year (Watson et al., 2018). However, this technology is quite costly and is not affordable for most research institutions in developing countries.

Continuous light from various sources has been shown to accelerate the reproductive period in most long-day plants (LDP) like pea (Berry and Aitken, 1979), spring wheat (Pérez-Gianmarco et al., 2020), chickpea (Sethi et al., 1981), and barley and radish (Turner et al., 2005; Stautas et al., 2011).

Hailstorms and heavy rains causing damage to wheat crop. This leads to almost complete damage of wheat crop in some LDP, wheat demands more photoperiod than the necessary length to flower. Most light sources have a spectrum that covers the photosynthetically active radiation (PAR) wavelength range of 400-700 nm. However, certain parameters, such as temperature, humidity, and lengthy daylight, are naturally available. Such environmental conditions can be advantageous for rapid generation advancement.

Taking more than one generation in a year under field conditions can be a sound strategy for wheat breeding as proved by CIMMYT using their world-famous shuttle breeding. However, in a country like India, two wheat crops in a year can be taken only using northern or southern hill stations of India during the monsoon (June-September) period.

The hill stations of India have limitation of space and suffer from years. Therefore, exploring suitability of an off-season crop in the plains of India could be a viable alternative to speed up the breeding process in low cost. In this study a location (Jabalpur) in Central India was tested for speed breeding under field conditions by growing wheat crop during off-season.

MATERIAL AND METHODS

Experimental site

The experimentation was done in the district of Jabalpur falling under the state of Madhya Pradesh. This region comes in the Central Plateau and Hills zone sub-region of Central Narmada Valley. Semi-arid climatic conditions persist throughout the region, with temperatures of 26°C to 40°C in July, 7°C to 24°C in January, and average annual rainfall from 50 cm-100 cm. Soils are mixed red, yellow, and black. Jabalpur falls in this agro-climatic zone.

The experiments were conducted at BISA farm, Jabalpur, Madhya Pradesh (23.18º N, 79.98º E) India using both normal and off-season. A similar experiment was conducted at BISA farm at Ludhiana, Punjab also.

Plant material

A total of five wheat genotypes including two popular varieties (MACS6222, GW322) and three advanced breeding lines (BWL6887, IND497, and GS3051 (GID: 7177664; GS\2015-16\3051; QUAIU#1//2*WHEAR/KRONSTAD F2004) were used for this study that was conducted in two consecutive crop years 2018 and 2019. Based on the promising results in the first year, the early maturing genotype GS1054 (GID: 6333158; GS\2015-16\1054; SUP152/BAJ #1) was also included in the experiment during the second year. The selected lines vary in genetic constitution and for days to maturity from early to normal in the central zone of India. The sowing was done on 16th Oct in 2017 in year 1 and 25th Oct in 2018 in year 2 following the recommended agronomic practices.

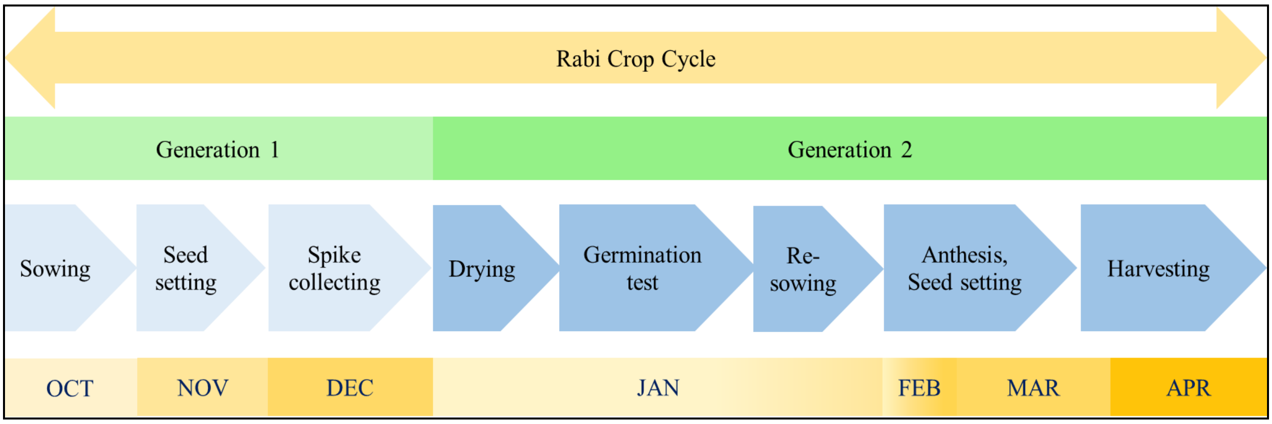
Field experimentation

The field experiment was conducted using four treatments to test and standardize the methodology as per the details given in Table 1. The data on phenological traits like days to heading (DH) and days to maturity (DM), days to anthesis (DA) and plant height

(PH) was recorded along with grain yield and thousand grain weight (TGW) from all the lines. The timelines of the activities from sowing to harvesting and growth stages for two cycles of generation advance are shown in Figure 1.

Table 1. Details of four treatments used in the experiments

Treatments	Spike collection time (DAF)	Drying in oven (Hours)
Treatment 1 (T1)	15	48
Treatment 2 (T2)	15	72
Treatment 3 (T3)	20	48
Treatment 4 (T4)	20	72



Rabi: The winter season for spring wheat that normally spreads from November to March in Central India.

Figure 1. Activities performed from sowing to harvesting in different months of the two cycles of generation advance implemented within one year

Treatment 1 (T1)

Five spikes were randomly collected from each genotype just before the early dough stage (15 days after Flowering; DAF). The selected spikes were sun-dried for nearly 6 hours, each for two consecutive days before placing them in the oven.

The spikes were kept in paper bags and dried in the oven for 48 hours at 35°C. Spikes were threshed by hand and seeds were shown directly in the field in two rows of 1.5 meters each with a line-to-line spacing of 22 cm.

Treatment 2 (T2)

The second treatment was like the T1 except that the spikes were dried in the oven for 72 hours at 35°C.

Treatment 3 (T3)

Five spikes were randomly collected from each genotype at the early dough stage (20 DAF). The selected spikes were sun-dried for nearly 6 hours before placing them in the electric oven at 35°C for 48 hours. The seeds were shown directly in the field in two rows of 1.5 meters each with a line-to-line spacing of 22 cm and seed-to-seed distance of approximately 8 cm.

Treatment 4 (T4)

The fourth treatment was like T3 with two modifications:

a) the spikes were dried in the oven for 72 hours at 35°C.

b) the seed was kept in Petri plates for germination (Figure 2b).

The seeds were moistened whenever needed. Germinated seeds were sown directly in the field under normal conditions.

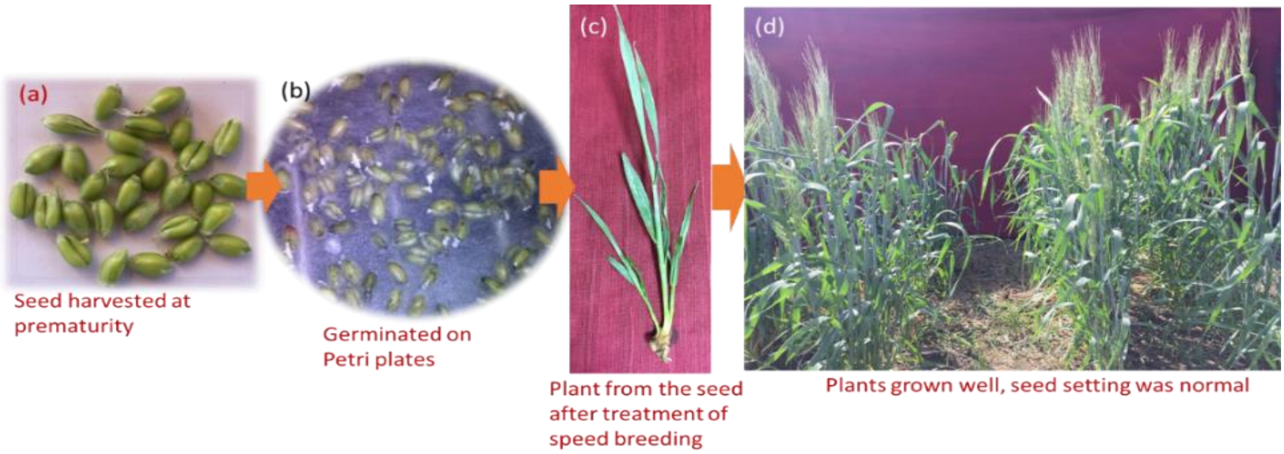


Figure 2. Stepwise demonstration of the experiments conducted, (a) seeds harvested just before early dough stage, (b) germinating seeds after sun and oven dry treatments, (c) seedling grown from the sown seeds, (d) fully grown plants

RESULTS AND DISCUSSION

Jabalpur is a high yield potential environment with a shorter duration than NWPZ and NEPZ (Figure 6). There was significant variation among genotypes for DH and DM (Table 2). The DH varied from 60 (MACS6222) to 70 days (GS3051) in the first cycle and 43 (GW322) to 55 (GS3051) in the second cycle of the first year, while grain yield and thousand grain weight were recorded only for the second cycle, where the range of the grain yield of the 189 gm (GW322) to 235 gm (GS3051) and range of TGW were 41.9 (BWL6887) to 47.2 gm (GS3051). Similarly, the DH varied from 55 (GS1054) to 74 (GS3051) in the first cycle and 35 (GS1054) to 54 (GS3051) in the second cycle of year 2, whereas the range of the grain yield of the 179 (BWL6887) to 242 gm (GS1054) and range of TGW were 39.0 gm (BWL6887) to 43.4 gm (MACS6222, GS1054). The weather conditions across two years did not show much variation (Figure 3a and 3b). Hence, there was no significant difference in the phenological traits of each of the lines.

Table 2. Observations recorded in the first and second cycles of the crop in the two years (2017-18 and 2018-19)

	Cycle 1				Cycle 2				Grain yield (0.6 m^2)	TGW (gm)
Genotypes	DH	DA	DM	PH	DH	DA	DM	PH		
First year (2017-18)										
BWL6887	63	69	129	78	49	54	94	74	190	41.9
IND497	65	69	130	101	51	53	93	89	209	46.4
MACS6222	60	65	130	94	45	50	92	79	228	49.9
GW322	60	66	126	87	43	46	91	76	189	47.7
GS3051	70	75	131	100	55	59	96	81	235	40.2
Second year (2018-19)										
BWL6887	62	68	132	80	50	56	96	75	179	39
IND497	66	70	130	103	50	54	95	88	215	41
MACS6222	59	65	131	92	43	47	94	78	236	43.4
GW322	59	64	127	85	43	44	92	77	196	42.1
GS3051	74	78	130	101	54	57	94	81	232	43.2
*GS1054	55	60	127	95	35	40	92	85	242	43.4

*The line 'GS1054' was included during the second year of the experiment;
DH = Days to heading; DA = Days to anthesis; DM = Days to maturity; TGW = Thousand grain weight.

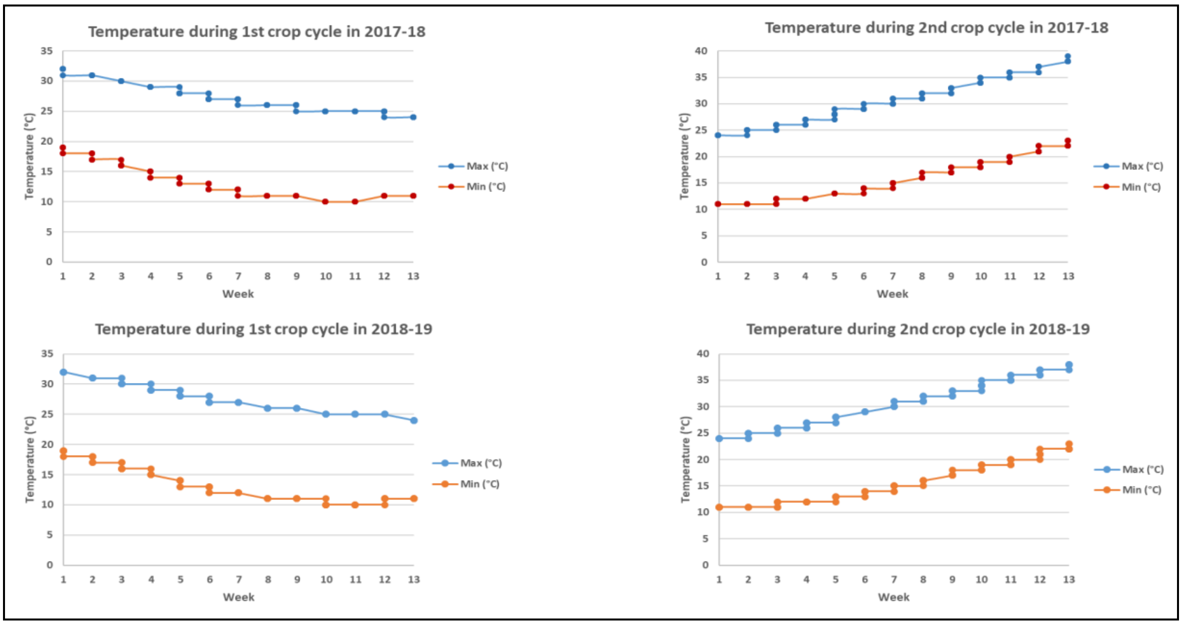


Figure 3a. Weekly temperature at BISA Jabalpur during experiment period covering both the cycles of a season (Oct-Apr in 2017-2018 and 2018-2019)

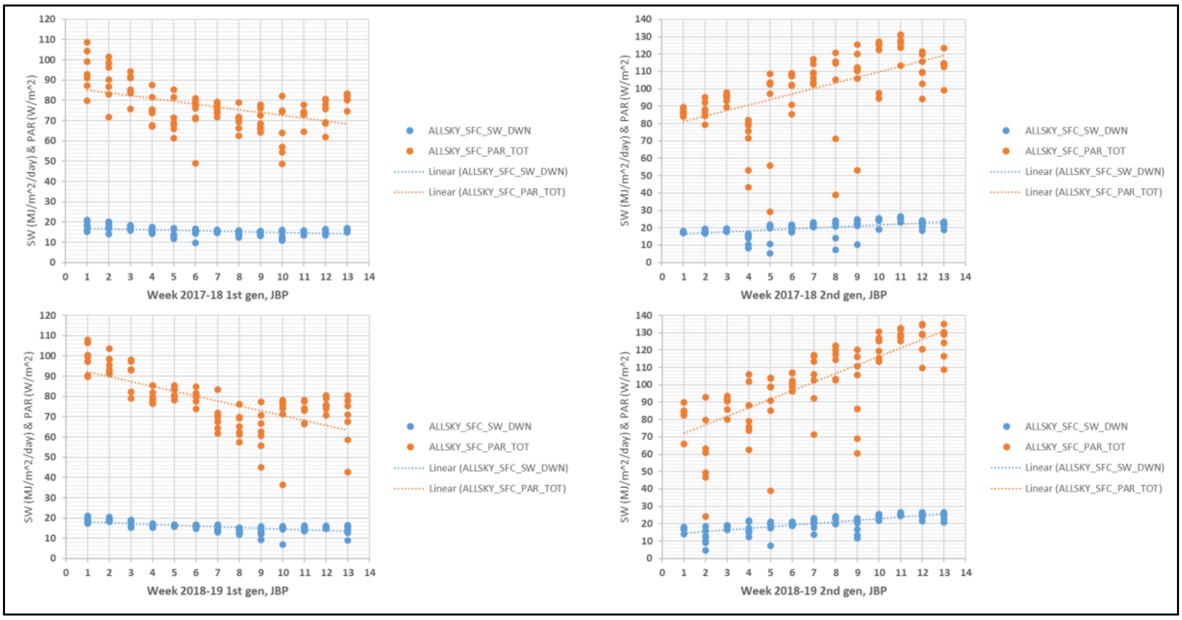


Figure 3b. Weekly solar radiation at BISA Jabalpur during the experiment period covering both the cycles of a season (Oct-Apr in 2017-2018 and 2018-2019)

All seeds failed to germinate in T1, while only a few germinated in T2 (Figure 4). However, T3 and T4 were successful in providing normal seeds from each of the genotypes. While T3 showed a partial success, the T4 was able to lead to normal seed development and germination in all cases. The average number of seeds retrieved from each spike ranged from 28-46. The seeds from the top and bottom of spikelets were not used due to low seed formation and too small grain size. The seeds from the individual spikes were dried and tested for germination. T1 seeds completely failed to germinate, while only a few seeds from T2 germinated. On the other hand, we observed nearly 80% and 98% germination in T3 and T4, respectively. The plots from T2, T3, and T4 of the second cycle in the field conditions are shown in Figure 4, while the healthy plants at ear emergence and maturity stages are shown in Figure 5.

Figure 3c. Drying the samples in the oven at 35°C

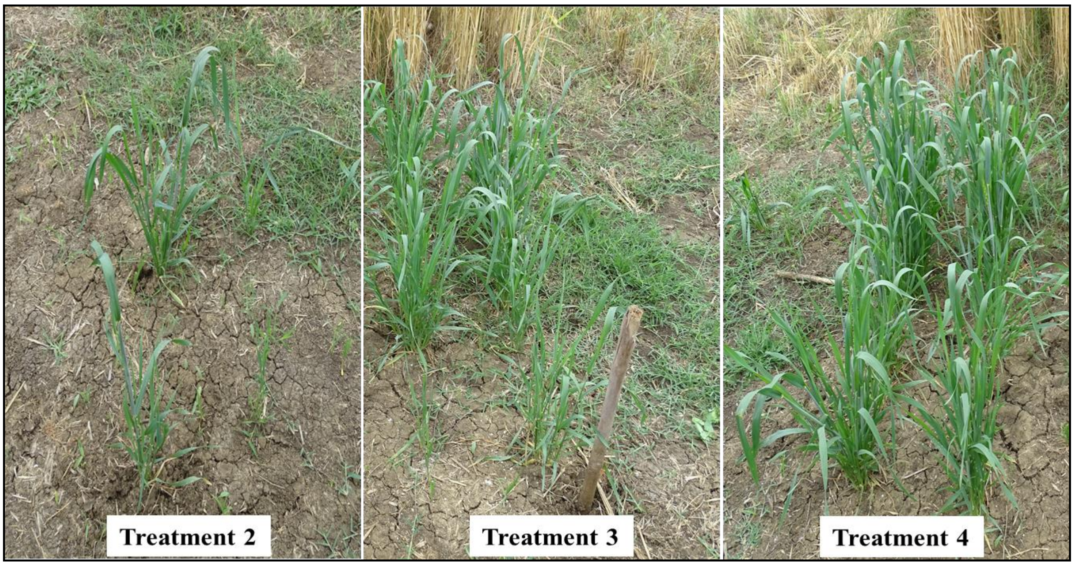


Figure 4. Germination of plants from T2, T3, and T4

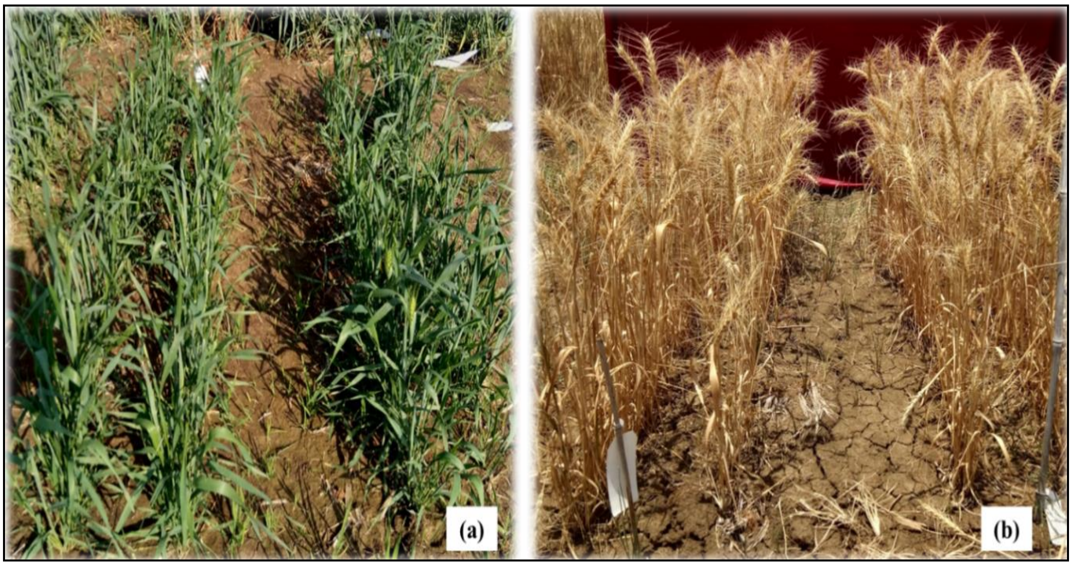


Figure 5. Plants from treatments 3 and T4 in a) flowering, and b) maturity stages

To test the hypothesis of the possibility of advancing generation twice in a year under field conditions in a location of India where off-season wheat is not grown and farmers and researchers take only one crop in a year, we evaluated the two most common varieties grown in the central zone along with four advanced breeding lines. Based on the first year of results at Jabalpur, the same experiment was also conducted at BISA Ludhiana (North-Western Plain Zones) to test the possibility of taking two generation of wheat crop in that location as well. To have common lines between Ludhiana and Jabalpur, 'GS1054 was included in the Jabalpur experiment in the second year of trials due to its suitability under zero tillage in NWPZ of India. However, the experiment at BISA Ludhiana was unsuccessful due to lower sunshine hours during December and January and longer crop duration.

The selected genotypes differed from each other for heading days, maturity days, and plant height. We tested diverse genotypes grown in the Jabalpur region to study whether the lines with different phenological traits and maturity durations may also be advanced by taking two crops in a year. Since the objective of this experiment was mainly to study the possibility and optimization of generation advancement, the thousand grain weight (TGW) gm, were recorded for the 1st cycle of the season, while grain yield were recorded to ensure the gain of sufficient seed for the next generation.

The experiment was conducted at BISA Jabalpur, which falls under the Central Zone of India and is a high wheat yield potential environment. The maturity of wheat takes place nearly at 168 days at Ludhiana (NWPZ) if planted early in October, and up to 158 days under normally sown conditions, i.e., when planted in the first week of November. Compared to Ludhiana, the crop duration at Jabalpur is about three weeks shorter, making this location ideal for growing/obtaining two generations within a year. The results suggest that it is possible to advance the generations at Jabalpur location by using the approach explained in this manuscript. However, it may not be feasible to take two crops for largescale seed multiplication or grain production since two crops methodology requires that ear heads will have to be harvested at dough or just before the dough stage. The grain remains succulent in these growth stages. Hence, there will be severe yield loss and deteriorated grain quality from a consumption point of view (Nedeva and Nikolava, 1999). However, for research purpose two crops can be taken in a year using the approach used in this study.

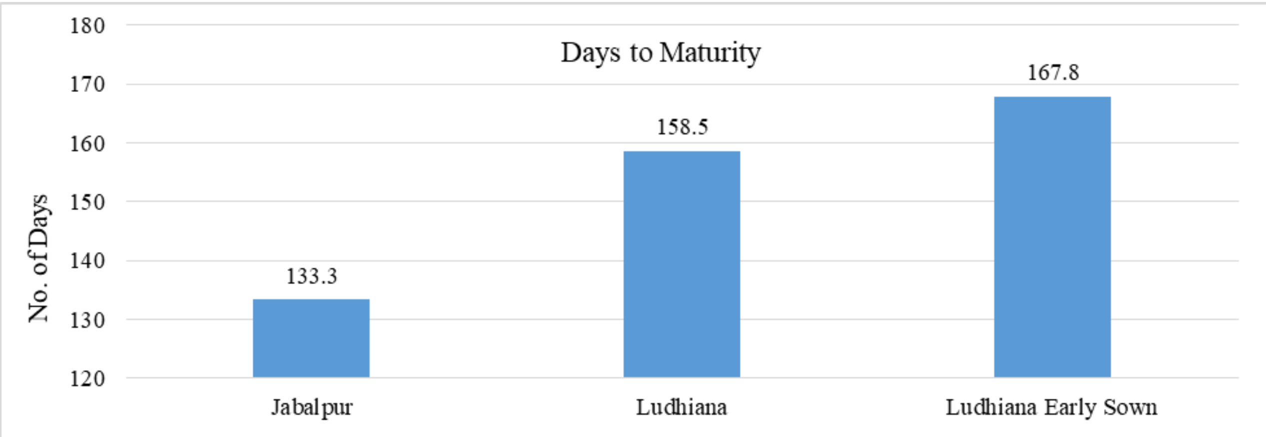


Figure 6. Average days to maturity at three BISA sites in three different wheat growing zones

The seed drying appears to have played a significant role, as evident from the first two treatments (T1 and T2) in which spike collection time was the same as in case of T3 and T4. Similar results proving that drying conditions are crucial for improving seed germination were reported by Nedeva and Nikolava (1999). Since the objective was to test the generation advancement in field conditions, the seeds were planted directly in the field. The third and fourth treatments were exercised five days after T1 and T2, where seeds were allowed to develop a bit more and attain the dough stage. As in T1, the seeds were dried for 48 hours in the oven at 35°C and planted directly in the field. Since we did not use seed from the topmost and lowest spikelets of a spike, the germination rate went up to 80%, just by providing five additional days for seed development. The germination further improved to about 100% using pre-planting germination in the Petri plates.

Based on the positive results obtained in the first year from T3 and T4, the same experiment was repeated in the next crop season except for T1 and T2. The additional high-yielding line GS1054 was also included in the experiment. The GS1054 (pedigree: SUP152/BAJ#1) is a relatively early maturing line with good yield performance and possesses tolerance to heat (Poudel et al., 2017). There was consistent seed germination in the second cycle (Figure 4). To ascertain the consistency in the protocol across the varieties, the agronomic characteristics like plant height, heading, maturity, growth habit, etc., were recorded (Table 2). There was no significant difference in phenological traits during the first crop cycle in both years. However, a reduction of 35 to 37 days in maturity was observed, which is expected, as has been reported earlier too (Rezaei et al., 2015; Poudel et al., 2017; Puri et al., 2020). All the genotypes selected for this study belong to the spring wheat group. Therefore, no vernalization was required, and plants could complete normal but shorter life cycles during the second cycle of generation advancement during the same year. A uniform trend of reduction in plant height, days to heading, and anthesis was observed in all the genotypes. These results showed almost similar impact of higher temperature on plant growth, but germination was unaffected.

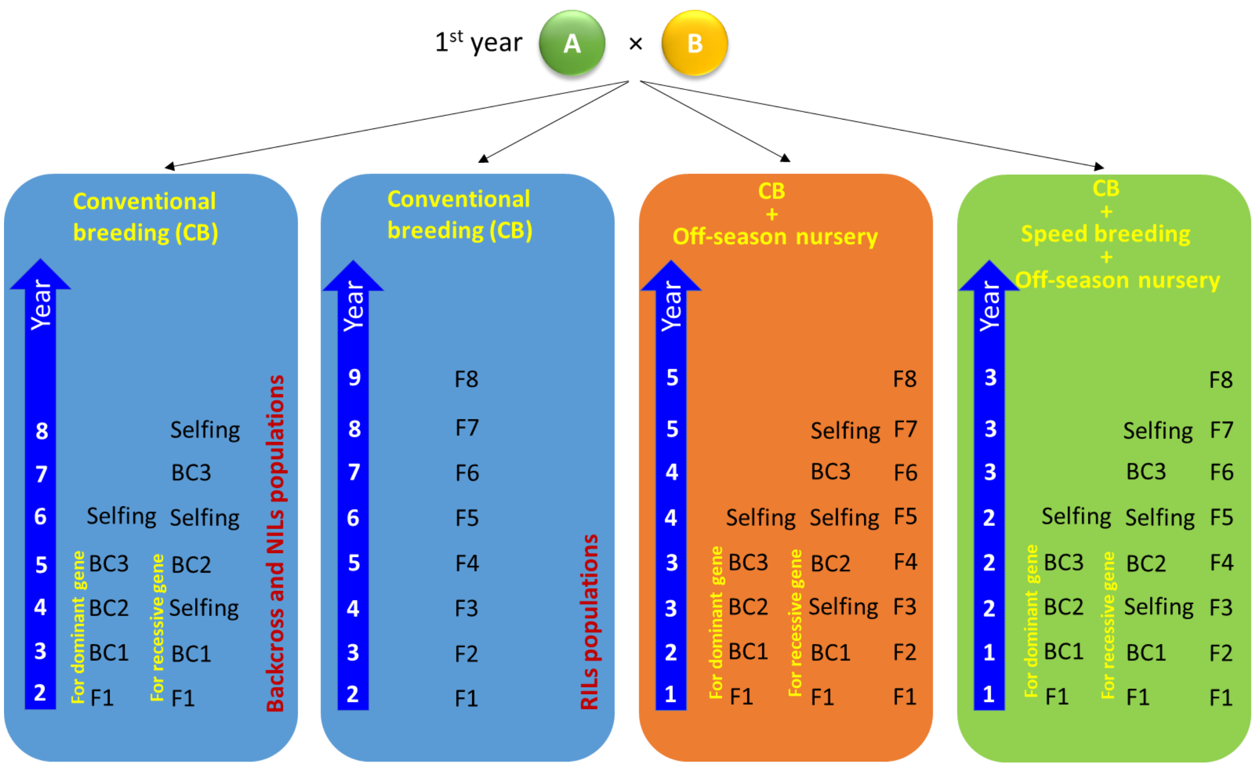


Figure 7. Different scenarios of following conventional breeding, speed breeding and speed breeding + off-season nursery

There was no significant difference in temperature between the first cycle of both years (Figure 5). The temperature during anthesis in the first crop cycle ranged from 11°C to 25°C. Notably, the flowering period of the second crop cycle was about 6-7 weeks, with a minimum temperature of 13°C and a max of 31°C in both years. It was observed that PAR was nearly 80 watts/m^2 during both seasons, making it ideal for growing two generations of wheat, mainly in the central zone of India. We observed almost a constant PAR value in both seasons. Therefore, the higher temperature appears to be the main factor in shortening the life cycle of plants. Several reports are commensurate with similar observations (Rezaei et al., 2015; Prasad et al., 2017; Liu et al., 2018, 2019; Shah et al., 2020). Along with the main rabi season if we go with off-season (May to September), there is potential to take three generations with minimal efforts and resources (Figure 7). This approach provides an advantage of selection in the segregating generation during the primary crop season, while seeds from the second cycle will increase the homozygosity in the population.

CONCLUSIONS

Based on the two years of results, we conclude that improving seed germination by different treatments led to the possibility to obtain two generations per season. This was possible in the central zone of India, where there are sufficient sunny days to support. The challenges to germinating premature seeds was also addressed by developing a new methodology. This Clubbing with off-season (May to September), there is potential to take three generations with minimal efforts and resources. The other potential application could be faster seed multiplication and dissemination of new varieties where the seed is available in limited quantity. The seed may be multiplied several hundred folds from a single grain in a season by harvesting heads (out of several tillers from a single plant) from the main wheat crop and planting in the available fields even in January.

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