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Development of somaclonal variants of wheat (*Triticum aestivum* L.) for yield traits and disease resistance suitable for heat stressed and zero-till conditions

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Abstract

In a substantial rice–wheat cropping system area of South Asia, wheat sowing often gets too delayed and exposed to terminal heat stress. Therefore, farmers prefer varieties that are able to perform well under a short growing period. Tissue culturally regenerated plants of wheat variety cv. HUW 234, the most widely cultivated variety of North Eastern Plain Zone (NEPZ) of India were screened using immature embryo as explant. Days to heading and maturity, yield and other yield components and resistance to leaf rust and spot blotch were evaluated. A few somaclones in R_3 and R_4 generations displayed significant earliness for days to heading and maturity, improved yield traits and resistance to leaf rust and spot blotch diseases. The superior performance of two of the variants was confirmed in the R_5 generation in 3 years of testing under two dates of conventional and zero-till sowing. Stability analysis also suggested superiority of the two somaclones across 12 environments. This appeared to confirm the possibility of obtaining useful somaclonal variants of wheat for very late sown as well as zero-till managed agriculture. The superior performing somaclones can be used as parents in the ongoing breeding programmes targeting late sown wheat in South Asia exposed to terminal heat stress.

Keywords: Tissue culture; Somaclonal variation; *Triticum aestivum*; Somatic embryogenesis; Zero till; Late sowing; Terminal heat stress

1. Introduction

Potential application of somaclonal variation in crops has been proposed as a supplementary tool to well established breeding approaches (Ahloowalia, 1982; Cheng et al., 1992; Ivanov et al., 1998). Plants regenerated from the embryogenic calli of wheat have revealed variants for various agronomic and quality characters such as plant height, stem thickness, leaf size, spike shape pollen fertility, gliadin storage protein, presence or absence of awns, maturity, plant type, etc. (Ahloowalia, 1982; Maddock et al., 1985; Carver and Johnson, 1989; Cheng et al., 1992). Somaclones have also been reported for various traits such as higher 1000 grain weight, protein concentration, sedimentation values, harder kernels (Hanson et al., 1994); plant height, spike length, main tiller diameter (Symillides et al., 1995; Ivanov et al., 1998); kernels per spike and kernel weight (Ryan et al., 1987; Mohmand and Nabors, 1990); resistance to brown rust (Tuchin et al., 1996; Rana et al., 1996); early maturity, high yield and superior quality (Liang et al., 1996); frost tolerance (Dorffling and Melz, 1997); resistance to scab caused by *Fusarium* spp. (Yang et al., 1998) and, higher yield, earliness and resistance to spot blotch (Arun et al., 2003). Apparently, there have been many reports on the isolation of somaclones for various traits in wheat. However, reports displaying agronomically useful variations of wheat suiting to late sown conditions and Resource Conservation Technologies (RCTs) such as zero till that are spreading fast in rice–wheat cropping areas of South Asia (Joshi et al., 2007a), are scanty.

The optimum time of wheat sowing in the eastern parts of Indo-Gangetic plains of Indian subcontinent is 15–25 November. However, in a substantially large area, especially that falling under 11–12 mha rice–wheat cropping system of South Asia (Hobbs and Morris, 1996; Pandey et al., 2005; Joshi et al., 2007a), wheat sowing often gets delayed primarily due to late harvesting of paddy (Joshi et al., 1997). This delayed sowing, which in around 4–5 million hectares goes even beyond third week of December, causes substantial loss to wheat yield due to terminal heat stress and lack of sufficient

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period for crop growth and development (Joshi et al., 2007b). Therefore, the farmers demand wheat varieties that are able to perform well under late sown conditions of rice–wheat cropping system. The other major problems of this zone are terminal heat stress and spot blotch and leaf rust diseases (Joshi et al., 2002; Pandey et al., 2005; Duveiller et al., 2005). Spot blotch disease caused by *Bipolaris sorokiniana* (sacc.) shoem syn. *Drechslera sorokiniana* (Sacc.) Subrm and Jain (syn. *Helminthosporium sativum*, teleomorph *Cochliobolus sativus*) is considered as no. 1 pathogen of wheat in eastern parts of South Asia encompassing India, Nepal and Bangladesh (Saari, 1998; Joshi et al., 2004a,b, 2007c,d). Superior performance of wheat varieties under zero-till sowings is being seen as an additional advantage since RCTs are expected to dominate rice–wheat cropping system areas of India in the coming future (Joshi et al., 2007a).

The present study reports the isolation of significantly superior variants of wheat variety cv. HUW 234 (the most popular cultivar of eastern Gangetic plains of South Asia under late sown conditions) for earliness, disease resistance and yield components under conventional as well as zero-till sowings. These variants can be used as donor for terminal heat tolerance that is gaining importance in view of the increasing threat of global warming.

2. Materials and methods

All the experiments were conducted at the research station of Banaras Hindu University, Varanasi, India (North Eastern Plains Zone, 25.2°N and 83.0°E). This centre falls under North Eastern Indo-Gangetic plains of India and has loam alluvial soil having neutral pH (7.2). In all the years of experiments, same agronomic practices recommended for normal fertility (120 kg N:60 kg P_2O_5:40 kg K_2O ha^{-1}) were followed. Full doses of K_2O and P_2O_5 were applied at the time of sowing. Nitrogen was supplied in split applications, 60 kg N ha^{-1} at sowing, 30 kg N ha^{-1} at the first irrigation (21 days after sowing) and 30 kg N ha^{-1} at the second irrigation (45 days after sowing). The previous crop in each year was paddy and the variety used was MTU 7029.

2.1. Tissue culture and plant regeneration

One of the most popular cultivars of eastern Gangetic plains of South Asia, cv. HUW 234 (HUW 12/CPAN 1666//HUW 12) was used as explant source. Selfed seeds from the nucleus seed plots were used as seed source. Immature embryos were cultured and plants were regenerated following the protocol described in our earlier study (Arun et al., 2003). The regenerated plantlets were referred to as R_0 (Hanson et al., 1994). In total, 389 R_0 spikes were harvested at maturity as single spike, to raise ear to row R_1 progeny.

2.2. Field evaluation of R_1 generation

The 389 R_0 spikes were screened in two sets of experiments. In the first experiment, single spikeline (R_1) of 2 m lengths were evaluated for yield traits and resistance to spot blotch along with parent as check. In the second experiment, single row of 1 m was sown separately for each R_1 to evaluate their resistance to leaf rust.

In the first set, the R_1 population was artificially inoculated with spot blotch pathogen *B. sorokiniana*, using most aggressive isolate (No. ICMP-13584; Auckland, New Zealand) identified at Varanasi, India (Joshi and Chand, 2002; Joshi et al., 2007c). Spot blotch was measured at growth stage 77 (Zadoks et al., 1974) using disease severities (%) as reported earlier (Joshi et al., 2004a,b). Area under disease progress curve (AUDPC) estimate was based on the plot disease severities at different growth stages (Van der Plank, 1963). Spot blotch severities (%) were assessed three times; at growth stage 65 (anthesis half complete to complete), 73 (early to medium milk) and 77 (early dough) (Zadoks et al., 1974). Area under disease progress curve (AUDPC) which is reported to be a pragmatic approach to measure resistance (Jeger, 2004) was calculated using following formula (Roelfs et al., 1992):

$$\text{AUDPC} = \sum_{i=1}^{a} \left[\left\{ \frac{Y_i + Y_{(i+1)}}{2} \right\} (t_{(i+1)} - t_i) \right]$$

where Y_i is the disease level at time t_i and $t_{(i+1)} - t_i$ is the time(days) between two disease scores.

In the second set that was planted for leaf rust evaluation, spreader rows of susceptible cultivar Agra Local were sown in the alleys and borders, 3 weeks before the sowing of experiment. For this disease, an artificial epiphytotic was created using race 77-5 which is the most virulent and frequently identified races from the Indian subcontinent (Nayar et al., 1994). The infector rows and the somaclonal variants were sprayed with 1 g of leaf rust uredospores suspended in 10 l of water with 2–3 drops of Tween 20 as dispersant solution. The field inoculations were done in the evening every third day till rust symptoms became visible on the susceptible spreaders. Field assessment of leaf rust severity at adult plant stage was based on a modified Cobb scale (Peterson et al., 1948).

On the basis of resistance to leaf rust and spot blotch, higher 1000 grain weight, earliness and yield components, 47 putative variants were selected for further evaluation in R_2 generation under very late sown condition, i.e., second week of January 1999.

2.3. Field evaluation of R_2 generation

Variants (R_2) were evaluated in a three sets of unreplicated trial along with the parent. First set was kept as protected by using fungicide Tilt at GS 54 and GS 69 (Zadoks et al., 1974) to prevent the attack of two common fungal diseases (leaf rust and spot blotch) and enable proper evaluation of yield traits. The other two experiments were conducted separately under artificial epiphytotic conditions for leaf rust and spot blotch diseases, respectively. Plot size for protected experiment was two rows of 2.5 m length, while for the other two experiments the plot size was kept single row of 2.5 m length. All the three sets were sown under very late sown (11 January 1999)