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Breaking the association between gametocidal gene(s) and leaf rust resistance gene (LrS2427) in Triticum aestivum-Aegilops speltoides derivative by gamma irradiation

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Abstract

Utilization of crop wild relatives of wheat can be very effective in building the genetic diversity to cater to the evolving strains of disease pathogens. *Aegilops speltoides* is a rich source of rust resistance genes however transferring those to wheat genome can be tedious due to co-transfer and preferential transmission of undesirable genes causing gametocidal activity. Such an unholy association was observed in *Triticum aestivum-Ae. speltoides* derivative line Sel. 2427 which possess the broad-spectrum leaf rust seedling resistance gene (*LrS2427*). In situ hybridization studies revealed the presence of S genome in Sel. 2427, showing translocations to six telomeric regions involving four chromosomes. This confirms the presence of genes associated with gametocidal activity along with the beneficial *LrS2427*. QTLs associated with the component traits of gametocidal activity have been localized to 2A, 2D, 3D chromosomes however candidate genes for fertility were identified only in 2A and 2D chromosomes. This is in accordance with the reports of gametocidal genes mapped to 2S chromosomes in *Ae. speltoides*. To break the inadvertent association, F_1(Agra Local X Sel. 2427) seeds were irradiated with gamma rays and leaf rust resistant mutants lacking gametocidal activity were developed. These mutants showed resistance to different races of leaf rust pathogen and showed superior agronomic performance as well. These mutants could be a great resource in wheat improvement for utilization of the leaf rust resistance gene *LrS2427* without any yield penalty.

INTRODUCTION

Crop wild relatives are a great source of unique alleles/genes to improve the available variability in the crop genepool. Wild relatives of wheat serve as valuable repositories of beneficial genes, and several rust resistance genes have been successfully incorporated into cultivated wheat varieties. *Aegilops speltoides* Tausch (2n = 2x = 14) belonging to the SS genome group, is considered as one of the progenitors of wheat. This species falls within the secondary gene pool and offers a promising source of novel valuable genes. *Ae. speltoides* genotypes possess a unique capability to facilitate homoeologous chromosome pairing when hybridized with wheat due to the presence of *Ph1* inhibitor or suppressor gene. *Ph1* suppressor genes in *Ae. speltoides* designated as *PhI* (Chen et al. 1994) and *Su1-Ph1* (7S) and *Su2-Ph1* (3S) (Dvorak et al. 2006; Li et al. 2017) has been transferred into wheat. This creates an opportunity for genetic exchange and the transfer of beneficial traits. Reports on transfer of traits especially disease resistance and heat tolerance (Awlachew et al. 2016; Seni et al. 2021) from *Ae. speltoides* to wheat are available in the literature. Majority of the traits targeted in *Ae. speltoides* are resistance to diseases like leaf rust, stem rust and powdery mildew, enhancing resilience against devastating fungal diseases. Out of 83 designated *Lr* genes, six genes *Lr28* (McIntosh et al. 1982), *Lr35* (Kerber and Dyck 1990), *Lr36* (Dvorak and Knott 1990), *Lr47* (Dubcovsky et al. 1998), *Lr51* (Helguera et al. 2005), *Lr66* (Marais et al. 2010b) have been introduced from *Ae. speltoides* into common wheat along with reports of some undesignated genes. Other genes derived from *Ae. speltoides* are *Sr32, Sr39, Sr47* in case of stem rust resistance and *Pm12* in case of powdery mildew resistance. Some of these alien introgressions were reported to be associated with undesirable traits. It was reported that introgression

of the alien chromosome segment involving *Lr47* gene was associated with detrimental effects on agronomic traits as well as on milling and baking-quality (Brevis et al. 2008). In case of stem rust, resistance genes *Sr32* and *Sr39* which are effective against the Ug99 race were transferred from *Ae. speltoides.* The introgression lines carrying *Sr32* and *Sr39* genes were not useful in wheat breeding program due to large *Ae. speltoides* segments which resulted in unintended yield penalty (Friebe et al. 1996). Those genes were useful only after shortening of the 2S chromosomal segments with *Sr32* and *Sr39* utilizing the *ph1b* mutant (Mago et al. 2009, 2013; Niu et al. 2011). Hence the utilization of beneficial genes from wild species presents a lot of challenges due to the associated genes causing linkage drag.

One such factor is the gametocidal gene(s) (selfish genes) present in some *Aegilops* sp. carrying S, S^{l}, S^{sh}, C and M genomes. Gametocidal factors have been reported in *Ae. speltoides* (SS), *Ae. longissima* ($S^{l}S^{l}$), *Ae. sharonensis* ($S^{sh}S^{sh}$), *Ae. markgrafii* or *Ae. caudata* (CC), *Ae. triuncialis* (CCUU), *Ae. cylindrica* (CCDD) and *Ae. geniculata* (MM) (Endo 1990; Tsujimoto 2005; Kishii 2019). *Gc* genes have been mapped in different species and are reported on the homoeologous groups: 2, 3, and 4 (Endo 2007; Kishii 2019). In *Ae. speltoides*, gametocidal genes have been mapped to 2S and 6S chromosomes (Tsujimoto and Tsunewaki 1984, 1988; Kota and Dvorak 1988). In *Ae. markgrafii* and *Ae. triuncialis*, 3C chromosome have been found to be responsible for gametocidal action (Endo and Tsunewaki 1975). The $4S^{sh}$ chromosome of *Ae. sharonensis* (Maan 1975; Endo 1985) was associated with gametocidal activity and the 'breaker' element (*GcB*) of the gametocidal locus was mapped to a region proximal to a block of sub-telomeric heterochromatin (Knight et al. 2015). Other chromosomes identified with gametocidal activity are 2C of *Ae cylindrica* (Endo 1979), $2S^{l}$ and $4S^{l}$ of *Ae. longissima*, $2S^{sh}$ and 4M of *Ae. geniculata* (Kynast et al. 2000). The severity of gametocidal genes vary in different species. For instance, in *Ae. speltoides* the gametocidal activity is grave, killing the gametes lacking gametocidal genes resulting in severe sterility and their preferential transfer. For instance, King et al. (2018) reported the preferential transmission of a 2S chromosome segment in all of the developed wheat-*Ae. speltoides* introgression lines due to its gametocidal activity. While, in species like *Ae. markgrafii*, it is mild, allowing incorporation of the gametes lacking gametocidal genes into progenies and it's possible to break the association in the segregating population (Rani et al. 2020). When introduced into the nuclear genome of wheat, these *Gc* genes induce sterility in gametes lacking the *Gc* gene(s), ensuring their preferential transmission. This results in semi-sterility in the F_1 generation (Endo 1979, 2007; Tsujimoto and Tsunewaki 1984). During interspecific hybridisation if accidental transfer of these gametocidal factors has occured along with the beneficial genes it becomes difficult to get rid of the sterility factors due to their preferential transmission. In those cases, the beneficial alien genes will be useful in breeding programmes only if such factors are eliminated. Some approaches were taken to alleviate the effects of gametocidal chromosomes. The effect of *Gc* gene is observed to be varied in different wheat backgrounds which suggests presence of suppressor genes for gametocidal activity. For instance, the *Gc* of chromosome 3C is normally lethal but in Chinese Spring background, its action is mild. Genes related to suppression of *Gc* genes was also reported in Norin 26 (designated as *Igc1*) in which *Ae. triuncialis Gc3-C1* action is inhibited (Tsujimoto and Tsunewaki 1985). Mutagenesis was also utilised to produce a mutant of the *Ae.*

sharonensis Gc2 gene (*Gc2mut*) which suppresses the effect of *Gc2*, which reduced the problems created by *Gc* genes in wheat breeding programmes (Friebe et al. 2003). For the removal of gametocidal genes from *Lr66* containing alien segment, allosyndesis was utilised in the S13introgression (multiple resistance to leaf, stripe and stem rust) which resulted in fertile recombinants with leaf rust resistance, however stem rust and stripe rust resistance genes were lost during recombination (Marais et al. 2010b).

As part of the distant hybridisation programme undertaken at Wheat Genetics & Cytogenetics lab, Division of Genetics, Indian Agricultural Research Institute, a *T. aestivum-Ae. speltoides* derivative line Selection 2427 (Sel. 2427) was developed. Previous investigations revealed that Sel. 2427 shows resistance to leaf as well as stem rust races (Dhakate 2002) and a single dominant gene, *LrS2427*, providing resistance against leaf rust was identified (Durgesh et al. 2011; Niranjana et al. 2017). Even though Sel. 2427 showed normal fertility in terms of pollen fertility and seed set, sterility was observed in the F_1 plants of Sel. 2427, when it was crossed to any wheat cultivar as shown by low pollen fertility and seed set. It was also observed that during meiotic metaphase, Sel. 2427 showed high frequency of PMCs (Pollen Mother Cells) with the normal 21^{II} (21 bivalents) configuration with exception of low frequencies of trivalent, quadrivalent, univalent and isochromosome indicating presence of alien translocation. Meiotic analysis in the F_1 generation showed drastic cytological instability with low frequency of PMCs with 21^{II} and increase in the number of univalents along with presence of trivalents, quadrivalents and isochromosomes (Niranjana et al. 2017). These observations serve as proof of typical gametocidal activity, and it suggests that alongside the leaf rust resistance gene, Sel. 2427 might also contain gene(s) responsible for gametocidal activity. Such an association complicates the utilization of this broad-spectrum resistance gene *LrS2427* in breeding programs. Hence this investigation was proposed with the following objectives: (1) Breaking the negative association between leaf rust resistance gene and gametocidal gene(s) in Sel. 2427 using gamma radiation (2) Identifying mutants with homozygous leaf rust resistance gene and good fertility parameters (3) Confirming the lack of gametocidal gene(s) in the selected leaf rust resistant mutants (4) Molecular cytogenetic characterisation of Sel. 2427 and (5) Locating genes/QTLs responsible for gametocidal activity using SNP markers.

MATERIALS & METHODS

Plant materials

Sel. 2427 is a hexaploid genetic stock developed by interspecific hybridisation using *Aegilops speltoides* (*T. aestivum* cv. Sonalika*2 / *Ae. speltoides* acc. No. EC331772/PI449338). Under simulated rust epiphytotic conditions, it was discovered to be resistant to leaf rust and stem rust (Dhakate 2002). Leaf rust resistance in Sel. 2427 was found to be regulated by a single dominant gene temporarily designated as *LrS2427* (Durgesh et al. 2011). However, utilization of *LrS2427* in wheat improvement was impeded due to associated gametocidal activity leading to partial sterility in crossed progenies as well as irregular meiotic configurations (Niranjana et al. 2017). Easy Thresh (ET) is an easy threshing sister line of Sel.

2427 also showing leaf rust resistance and partial sterility. The mapping population F_2(Agra Local X Sel. 2427) was developed for identifying QTLs associated with component traits of gametocidal activity.

Development of leaf rust resistant mutants lacking gametocidal activity

Gamma ray mutagenesis was undertaken at F_1 generation instead of Sel. 2427 itself, since phenotyping for gametocidal activity can be done in the heterozygous state. One hundred F_1 (Agra Local X Sel. 2427) crossed seeds each were treated with 250 Gy and 300 Gy gamma radiation using Gamma Radiation Chamber (GC 5000) at National Research Laboratory (NRL), IARI. The M_0F_1 plants were planted during Rabi 2017-18 and data was recorded on number of seeds and spikelets per spike. From the data two parameters were estimated to ascertain fertility *viz.*, average seed set per spike (ASS) and percentage of empty spikelets per spike (PES). The best fertile M_0F_1 plants were identified and carried forward to develop homozygous leaf rust resistant mutants. At M_3F_4 and M_4F_5 stages, 161 homozygous leaf rust resistant mutants were subjected to replicated field trial to study fertility parameters along with 39 control genotypes *viz.*, Sel. 2427, ET, homozygous leaf rust resistant RILs of Sel. 2427 (ALS2427) & ET (ALET) and susceptible lines Agra Local (AL), Sonalika, HD2932. Comparing the fertility data best mutants were identified. To confirm the loss of gametocidal activity these mutants were also pollinated using HD2932 and F_1 generation was analysed for fertility traits. The scheme of development of leaf rust resistant mutants lacking gametocidal activity is demonstrated in Fig. 1.

Field experiment and statistical analysis

For fertility studies of the mutants, 200 genotypes including gamma mutants (161), ALS2427 RILs (20), ALET RILs (14), susceptible control (3) genotypes and resistant control genotypes (2) were sown in 1m lines. For design of the experiment Randomised Block Design (RBD) was used with three replications. During *Rabi* 2020-21, the homozygous resistant mutants at M_3F_4 stage along with control genotypes were sown in RBD trial. The best plants in each mutant series were selected based on lowest percentage of empty spikelets and carried over to next year. During *Rabi* 2021-22, the best plants in each mutant series at M_4F_5 stage were analysed in RBD trial. The statistical model used was:

$y_{ik} = \mu + b_k + g_i + g_{ik}$, i = 1,. . ., g; k = 1,. . ., r

where yik is the observation of the i^{th} genotype in the k^{th} replication, g is the total number of genotypes, r is the number of replications/blocks

For raising the mapping population F_2(Agra Local x Sel. 2427), 700 seeds were sown. Out of 700, only 519 plants were germinated (Suppl. Figure 3). Out of 519, only 496 plants reached spike emergence stage. Since F_2 is a mortal population, it cannot be replicated. Hence augmented design was applied using Agra Local and Sel. 2427 as the replicated control plants. ANOVA for augmented design was

calculated using SPAD (Statistical Package for Augmented design) software. The statistical model used was:

$Y_{ij} = \mu + \beta_i + C_j + \tau_k(_i) + \varepsilon_{ij}$

where, μ = mean, β = blocks, C = checks, τ = new entries, ε = error

Estimation of fertility parameters

For mapping of gametocidal activity in F_2 population, four component traits were chosen viz., average seed set per spike (ASS), percentage of empty spikelets per spike (PES), average pollen fertility % (APF) and seed shrivelling index (SSI). For fertility analysis in the M_0F_1 generation as well as F_2 mapping population, ASS and PES were estimated after harvesting all spikes in the plant using the following formulae given below. Whereas in case of fertility trial experiment of gamma mutant lines, five spikes were harvested per line based on which ASS and PES were estimated. In the F_2 mapping population, APF was calculated for each individual plant. Another trait seed shrivelling was also prevalent in the F_2 mapping population. Number of seeds which are bold, partially shrivelled and completely shrivelled are counted for each plant and SSI was calculated.

ASS = ΣS/N, where S = No. of seeds in one spike, N = Total no. of spikes per plant

PES = Σ [{(Sp x 3)-S}/3]/Sp x 100, where Sp = No. of spikelets in one spike, S = No. of seeds in one spike

APF = Σ (F/T)/3 x 100, where F = No. of fertile pollen grains, T = Total No of pollen grains (Data recorded on anthers from lower, middle and upper portions of spike.)

SSI= {(B x 0) + (PS x 1) + (CS x 2)}/T, where B = No. of bold seeds, PS = No. of partially shrivelled seeds, CS = No. of completely shrivelled seeds, T = Total no. of seeds

Genomic in situ hybridisation (GISH) and Genomic dot blot assay

The probe for GISH was prepared from whole genomic DNA of *Ae. speltoides* using FISH Tag™ DNA Kit (Catalogue no: F32947). DNA isolation and purification was done from young leaves by CTAB method. In the first step, DNA was subjected to nick translation followed by purification and ethanol precipitation. In the second step labelling of the probe was done by dye coupling with Alexa Fluor 488 dye (492/520 nm). Slides were prepared using root tip meristematic tissues for mitotic chromosomes and anther tissues for meiotic chromosomes. Slide pre-treatment, denaturation, hybridisation and post hybridisation washes were carried out based on existing protocols with slight modifications (Barch et al. 1991; Schwarchzacher and Heslop-Harrison 2000). For visualisation, GISH signals were captured using the FITC filter (Excitation peak at 491 nm; emission peak at 516 nm), since the probe is tagged to the fluorescent dye Alexaflour 488 which is spectrally similar to FITC (Fluorescein isothiocyanate). Since DAPI (4,6-Diamidino-2-phenylindole) was used as the counter stain, images were captured using DAPI

filter (excitation peak at 359 nm; emission peak at 457 nm) to visualise chromosomes. Hence, two images of the field of view were captured and merged to create a complete image using Nikon (Nikon Eclipse Ni series) epifluorescence microscope.

For genomic dot blot analysis Digoxigenin labelled probe was developed from whole genomic DNA of *Ae. speltoides* using DIG-Nick Translation Mix (Cat. No. 11745816910, Roche). The DNA of positive control (*Ae. speltoides*), negative control (Sonalika) and *Ae. speltoides* derivatives *viz.*, Sel. 2427 and ET was blotted on nylon membrane (Invitrogen AM10102) at concentration 200ng/µL. Dot-blot genomic hybridisation was carried out based on the procedure given by Rey et al. (2017b) with minor modifications (Suppl Fig. 1).

SNP genotyping and analysis

High quality genomic DNA of parental genotypes (Sel. 2427 & Agra Local), 64 F_2 plants, 12 mutants, Sonalika and ET was isolated using CTAB method and quality was ascertained by checking using Nanodrop spectrophotometer and electrophoresis using 0.8% agarose gel (Verma et al. 2020). SNP Genotyping was conducted using Axiom 35 K Breeder's SNP Array (Imperial Life Sciences, Gurgaon, India). Genotyping calls for 35,143 SNP markers were obtained, which were filtered in a series of steps. Only those SNPs which showed polymorphism between two parents were utilized for linkage map construction. SNPs lacking any chromosome ID and position were removed. Polymorphic markers having more than 10 missing values for the RILs were also filtered out. These markers were tested for significant segregation distortion using Chi-square test. Markers with $p < 0.01$ chi-square test for segregation distortion were removed thus keeping only the high-quality SNPs using the NIN function of QTL IciMapping. Finally, 4925 SNPs were further carried to linkage grouping using Kosambi method and LOD score as 3.0 with the help of QTL IciMapping v.4.2 (Meng et al. 2015). Using 4925 SNPs, QTL mapping studies were conducted with Inclusive Composite Interval Mapping of ADDitive (ICIM-ADD) in QTL IciMapping software for 21 linkage groups and 4 traits (Meng et al. 2015).

For clustering of the 12 gamma mutants along with four control genotypes, UPGMA clustering approach was computed using TASSEL (version 5) (Bradbury et al. 2007). 8429 polymorphic SNPs were utilized for carrying out phylogenetic analysis (Fig. 3c). In the obtained clusters, further editing was done using iTOL v6. (*Interactive Tree Of Life) which is an online tool for editing, annotation and displaying phylogenetic trees* (Letunic and Bork 2021).

Pathogen and inoculation

The gamma mutants were screened for seedling resistance against leaf rust race 77 − 5 at M_1F_2 as well as M_2F_3 generation to identify homozygous resistant families. The gamma mutants selected after fertility analysis were subjected to Single Race Testing (SRT) using eight different races *viz.*, 77 − 5, 77 − 2, 77 − 6, 77 − 8, 77A-1, 12 − 5, 106 and 162A. Pure inoculum was sourced from the Regional Station of the Indian Institute of Wheat and Barley Research (IIWBR), Flowerdale, Shimla. The rust inoculum underwent multiplication using the susceptible variety Agra Local within a controlled glasshouse setting.

For studying seedling or All Stage resistance (ASR), the seedlings were raised in trays. After 10 days of sowing the seedlings were ready for inoculation. The multiplied inoculum was mixed with water containing one drop of Tween20 and agitated vigorously. Using a sprayer, the inoculum was evenly spread on seedlings. The trays were incubated for 48 hours in a humidity chamber and was kept for disease development. Disease reaction appeared roughly around 12 days and scoring was done (Stakman et al. 1962).

Evaluation of yield parameters of gamma mutants

A field experiment was conducted with mutants and control genotypes planted in rows measuring 1 meter in length. Observations were recorded for five (n) individual plants within each genotype. Various yield associated traits, *viz.*, plant height, the number of tillers, the number of spikelets per spike, and the number of seeds per spike, were meticulously documented. Subsequently, statistical analyses were employed to compute the mean values and standard deviations for each trait. These statistical measures were then subjected to comparative analysis to assess differences and variations among the genotypes.

Mean = $\Sigma x_i/n$

Standard deviation, $\sigma = \sqrt{(\Sigma(x_i - \mu)^2 / n-1)}$

Where x_i is the observation for i^{th} plant, μ is the mean and n is the total no of plants (= 5)

Meiotic analysis of leaf rust resistant and fertile gamma mutants and their F_1 generation

Immature spikes of the mutants and F_1 plants were harvested at booting stage and fixed in Carnoy's I fixative and stored in 4^0C. Anthers with PMCs at meiosis I, particularly meiotic metaphase I were identified. Selected anthers were hydrolysed in 1N HCl solution at 60^0C for 12 minutes. It was then transferred to the prepared Feulgen solution and kept in the dark for at least 1 hr. Slides were prepared using the stained anthers by squash method using 2% acetocarmine (Niranjana et al. 2017).

RESULTS

Effect of gamma ray mutagenesis on F_1 (Agra Local x Sel. 2427) plants

Out of 100 M_0F_1 seeds treated with 250 Gy gamma radiation (T250), only 63 plants survived beyond spike emergence stage. Whereas out of 100 M_0F_1 seeds treated with 300 Gy gamma radiation (T300), 64 plants survived beyond spike emergence stage. For comparing the fertility parameters of the gamma ray treated F_1 plants, 27 untreated F_1 plants were also raised side by side as control. ASS in the control

plants ranged from 1.75 to 10 with a mean value of 5.84. In the T250 plants, ASS ranged from 0 to 24 and in T300 plants, it ranged from 0 to 30.6. PES in the control plants ranged from 80.56 to 95.98 with an average of 89.49. In the T250 plants, PES ranged from 38.46 to 100, whereas in case of T300 plants, it ranged from 39.28 to 100. Based on the higher average seed set value and lower empty spikelet percentage, the F_1 plants T250-20, T250-42, T250-34, T250-18, T250-22, T250-26, T250-81, T300-7, T300-67, T300-17, T300-37, T300-40, T300-50, T300-64, T300-65, T300-68 were carried forward to next generation (Fig. 2).

The seeds harvested from control F_1 plants can be categorized into bold, partially shrivelled and completely shrivelled (Fig. 2a). While comparing the control F_2 seeds and the irradiated M_1F_2 seeds it was observed that notably in mutants T250-20 and T300-67 (Fig. 2b) there was no shrivelled seeds. While in case of other selected mutants M_1F_2 there was lower number of shrivelled seeds as compared to control F_2 seeds (Fig. 2c). The M_1F_2 plants were screened for leaf rust seedling resistance and plants showing high level of leaf rust resistance (;1) were transplanted (Fig. 2d) and plants showing 'X' reaction (;13) as well as susceptibility (3) were rejected. While harvesting the M_1F_2 plants, weak and less vigorous plants with low seed set were discarded so that the M_2F_3 plants were mostly robust. The M_2F_3 families were screened for leaf rust seedling resistance and homozygous resistant families were identified and carried forward to next generation as single plant selections. Again, while harvesting M_2F_3 plants only robust plants with good seed set were preferred. Finally, the selected best 161 M_3F_4 lines were analyzed for fertility parameters in field experiment.

Fertility analysis of homozygous leaf rust resistant gamma mutants lacking gametocidal activity

During Rabi 2020-21, the 161 gamma mutants at M_3F_4 stage were raised along with the 39 control genotypes in RBD design. It was noted that the mutants showed uniform growth and good plant stand as compared to the control RILs *viz.*, ALS2427 and ALET which exhibited poor germination rate and less uniformity within lines. Five spikes per line were harvested and data was recorded on number of seeds and spikelets. ASS and PES were estimated for each line. During Rabi 2021-22, the experiment was repeated. ANOVA for both experiments is given in the supplementary information (Suppl tables 1 & 2). ASS of the mutants ranged between 22.33 to 59.33 and 21.07 to 56.97 in 2020-21 and 2021-22, respectively. While in case of control, the range was 20.27–46.93 and 10.47–52.93. PES in the mutants ranged from 4.73 to 55.24 and 0.12 to 54.20 in 2020-21 and 2021-22, respectively. Whereas in case of control, the range was 17.31–65.22 and 3.53–73.81. In the first year, highest ASS over three replications was recorded in T300-67-6-4 (59.33) and in second year it was T300-67-6-5 (56.97). In the first year, T300-67-64-77 showed the lowest PES (4.73) and in second year it was T300-67-116-6 (0.12).

A subset of the selected mutants along with control genotypes was used as pollen parent to cross HD2932 to develop F_1 generation to confirm the loss of gametocidal activity. Data on fertility traits were also recorded in the individual F_1 plants and compared with those of the parent mutant (Fig. 3). In the

control genotypes, ASS ranged from 23 in ET to 38.8 in ALS2427-138-8, while in the F_1 generation, it plummeted to range of 6.73–15.87. In case of PES, control genotypes showed a range of 14.24 to 51.66. While in the F_1 generation, PES increased to the range of 71.52 to 87.57. In case of the mutants, ASS ranged from 29.6 in T300-67-113-4-1 to 54.07 in T250-20-20-6. While in the F_1 generation, it was observed that ASS was comparable with the parents showing a range of 32.2 to 66.3. PES of the mutants ranged from 4.62 in T250-20-20-6 to 40.14 in T300-67-113-17 and in the F_1 generation, it ranged from 0 to 44.73. This graph shows a clear picture on the improvement in fertility traits in the mutants even in F_1 generation. Seed shrivelling which was observed in the F_1 generation of the control genotypes was not observed in case of mutants from M_1F_2 generation itself as well as after crossing (Fig. 4a,b). Hence it is safe to conclude that these mutants lack gametocidal gene(s).

Single Race Testing of the best mutants

The selected 21 gamma mutants exhibited a notable level of seedling resistance to eight distinct leaf rust races, encompassing even the highly virulent and prevailing race, 77 − 5. The findings from the evaluation of these gamma mutants against various leaf rust races are elucidated in Table 1. The mutants displayed resistant responses across different races, showcasing infection types (ITs) ranging from IT '0' to IT ‘;1+’ (Fig. 3d). In contrast, HD2932 and Agra Local displayed susceptibility (ITs '3' to '3+') while Sel. 2427 and ET showed resistance (ITs ;1 to 11+) to all the races examined.

Table 1
Multi pathotype testing of Gamma mutants at seedling stage

Sl. No.	Genotype/Mutant	77 – 8	12 – 5	77 – 5	106	77A-1	77 – 6	162A	77 – 2
1	Sel. 2427	-	-	;1	1+	;1	-	;1	11+
2	ET	;	;n	;1-	;	;1-	;1-	;1-	;
3	HD2932	3	3	3	3+	3	3	3	3
4	Agra Local	-	-	3	3	33+	-	3	3
5	T250-20-20-6	;	;n	;	;	-	;	;3	0
6	T250-20-20-16	;	-	;1	-	-	-	;	;
7	T250-20-44-3	;	;	;	;	;	;	;	;1
8	T250-20-12-2	;	;	;	;	;	;	;	;
9	T250-20-12-4	;	;	;	;	;1	;	;	;1
10	T300-67-64-60	;	;	;n	;	;	;1-	;	;
11	T300-67-64-4	;	;n	;	;	;n	;	;	;
12	T300-67-64-67	;	;n	;	;	;	;	;	;1-
13	T300-64-51-1	;	;n	;	;	;	;	;	;
14	T300-64-51-28	;	;n	;	;	;	;1+	;	0
15	T300-64-70-7	;	;n	;	;	;	;	;	;
16	T300-67-6-4	;1--	;n	;1-	;	;	;	;	;
17	T300-67-6-6	;	;n	;1-	;	;n	;1-	;11-	;
18	T300-67-102-11	;	;n	;n	;	;n	;	;1	;
20	T300-67-116-12	;1-	;n	;n	;	;	;1	;1-	;
21	T300-67-116-12-1	;	;1-	;	;	;	;1-	;1-	;
22	T300-67-137-14	;	;	;	;	;n	;11-	;	;
23	T300-67-113-5	;	;	;n	;n	;n	;1-	;	;
24	T300-67-136-1	;	;	;	;	;n	;	;	;
25	T300-67-101-4	;	;n	;1-n	;1-	;n	;11-	;1	;

Molecular cytogenetic characterisation of Sel. 2427

For genomic in situ hybridization in Sel. 2427 the meristematic tissues of the root tip were used. Positive fluorescence signals were observed in 158 cells. Out of 158 cells, three showed one signal, seven

showed two signals, 19 showed three signals, 25 showed four signals, 39 showed five signals and 61 showed six signals (Table 2). While very few cells showed more than six signals. Consistent presence of six fluorescence signals in the chromosomes of most cells (Fig. 5) points to the fact that three introgressions might have happened between *Ae. speltoides* (S) genome and wheat genome (AABBDD). It was also observed that in one pair of chromosomes, *Ae. speltoides* introgressions have happened in both the telomeric regions and in another pair of chromosomes, S genome introgression is limited to one telomeric end. Hence the three introgressions transferred from *Ae. speltoides* involves two pairs of chromosomes resulting in six GISH signals.

Table 2
Number of cells showing different number of GISH signals in Sel. 2427 and F_1(Agra Local × Sel. 2427)

Tissue	Genotype	Total number of cells observed	Number of cells with 'n' number of GISH signals						
			n = 1	n = 2	n = 3	n = 4	n = 5	n = 6	n > 6
Root	Sel. 2427	158	3	7	19	25	39	**61**	4
Anther	F_1(AL×Sel. 2427)	1013	46	291	**609**	57	7	2	1

To support the findings additional genomic in situ hybridization experiments were done in the anther tissues fixed from the plants of F_1(Agra Local × Sel. 2427). The F_1 plant will be having half of the chromosomes from Sel. 2427 and another half from Agra Local. Positive fluorescence signals were observed in 1013 cells. Out of 1013 cells, 46 showed one signal, 291 showed two signals, 609 showed three signals, 57 showed four signals, seven showed five signals, two showed six signals and one showed more than six signals (Table 2). In support to our earlier findings, there was consistent observation of three fluorescence signals in the chromosomes of majority of cells (Fig. 6). In some PMCs, two signals were closely placed indicating the chromosome with two telomeric regions from *Ae. speltoides*. While in some PMCs two signals were far apart which may be due to occurrence of crossing over during pachytene.

QTL mapping of component traits for gametocidal activity

QTLs identified for the four component traits for gametocidal activity *viz.*, ASS, PES, APF and SSI are summarised in Table:3. For the component trait ASS, four significant QTLs were detected in the chromosomes 2A, 3A, 3D and 4A. LOD scores of these QTLs ranged from 3.06 to 4.89. Phenotypic variance explained (%) varied from 5.99 to 15.10. Out of these four, two QTLs on chromosome 2A and 3D exhibited high PVE (%) *viz.*, 13.1533 and 15.101, respectively. For the trait APF, 13 QTLs were detected. LOD scores of these QTLs ranged from 3.14 to 4.17. Phenotypic variance explained (%) was low for all the QTLs which ranged from 2.12 to 6.26. For the component trait SSI, eight QTLs were identified with LOD scores ranging from 3.09 to 10.37. Phenotypic variance explained (%) varied from 4.28 to 21.70. QTLs explaining highest phenotypic variance was located on chromosomes 2A (15.03%) and 2D

(21.70%). For the trait of PES, four QTLs were detected. LOD scores of the QTLs ranged from 3.08 to 5.66 explaining phenotypic variance in the range of 6.02–14.78%. QTLs explaining highest phenotypic variance was located on chromosomes 2A (14.78%) and 3D (13.88%).

From the frequency distribution graph (Suppl. Figure 3), it was evident that all four traits showed skewed distribution suggesting that the genes/QTLs responsible have major effects. Hence in the results, the QTLs explaining high levels of phenotypic variance alone are discussed further. In the 2A chromosome, QTLs were detected for the traits ASS (PVE = 13.15%) and PES (PVE = 14.78%) between the markers AX-*94961148* and *AX-94399039*. In the nearby loci a QTL for SSI (PVE = 15.03%) was also detected, however in a different marker interval *AX-94585454 – AX-94407698.* In the 3D chromosome, QTLs were detected for traits ASS (PVE = 15.10%) and PES (PVE = 13.88%) between the markers AX-*95098367* and *AX-95091443.* In the 2D chromosome, a major QTL was observed for SSI (PVE = 21.70%) in the marker interval *AX-94830689* and *AX-94783818*. Except average pollen fertility all other component traits showed QTLs with high PVE which suggests that seed shrivelling, average seed set per spike and empty spikelet percentage are the better component traits for studying gametocidal activity.

Table 3
QTLs identified for the traits ASS, APF, SSI and PES

Trait	Chr No.	Position	Left Marker	Right Marker	LOD	PVE (%)
Average seed set per spike (ASS)	2A	643	AX-94961148	AX-94399039	4.89	13.15
	3A	212	AX-94565216	AX-94567027	3.18	6.20
	3D	71	AX-95098367	AX-95091443	3.97	15.10
	4A	822	AX-94916776	AX-94913649	3.06	5.99
Average Pollen fertility percentage (APF)	1B	54	AX-95025706	AX-94766462	4.05	2.29
	1D	146	AX-95153822	AX-94435033	3.87	5.84
	3A	216	AX-94565216	AX-94567027	4.12	3.62
	3B	1592	AX-94392034	AX-94754467	3.19	2.12
	3D	25	AX-94855154	AX-94740478	3.19	2.14
	4D	721	AX-94744774	AX-94479174	3.14	6.26
	5A	1304	AX-94793259	AX-94560945	3.18	2.82
	5A	1329	AX-94560945	AX-94455495	4.17	5.91
	5A	1350	AX-95208994	AX-94401553	4.08	2.30
	5D	1104	AX-94838278	AX-94595328	3.34	5.97
	7A	393	AX-94749760	AX-94708164	3.35	5.95
	7B	1512	AX-94434165	AX-94419416	3.15	2.67
	7D	223	AX-94680011	AX-95139334	3.22	5.51
Seed shrivelling index (SSI)	1A	516	AX-	AX-	4.71	5.62

Trait	Chr No.	Position	Left Marker	Right Marker	LOD	PVE (%)
			94450466	94977486		
	2A	246	AX-94684375	AX-94717890	3.38	4.28
	2A	694	AX-94585454	AX-94407698	7.40	15.03
	2B	112	AX-94860003	AX-94571927	3.09	5.00
	2B	1501	AX-94993660	AX-94937945	3.85	8.16
	2D	1810	AX-94830689	AX-94783818	10.37	21.70
	3B	1432	AX-94870174	AX-94404804	4.91	8.24
	4D	303	AX-94992713	AX-94392424	3.90	9.50
Percentage of empty spikelets (PES)	1D	495	AX-94514240	AX-94538909	3.08	6.02
	2A	643	AX-94961148	AX-94399039	5.66	14.78
	3B	147	AX-95003876	AX-94570222	3.22	6.02
	3D	73	AX-95098367	AX-95091443	3.51	13.88

Candidate Gene Identification

Candidate genes for ASS, PES and SSI were predicted in the major QTLs discussed earlier. In the 2D chromosome, a major QTL was observed for SSI in the marker interval *AX-94830689* (595167666) and *AX-94783818* (591948766). Sixty two putative gene(s) for seed shrivelling were predicted in this 3.2 Mb long marker interval. These 62 genes belonged to different classes showing different gene functions *viz.*, Xyloglucan endotransglucosylase/hydrolase, mediator of RNA polymerase II transcription subunit 7 and 13, protein Pelota homolog, molybdate transporter 2 (MOT2), amylogenin, glutamine synthetase, transmembrane 9 superfamily member, molybdopterin biosynthesis protein CNX1, 2-methoxy-6-polyprenyl-1,4-benzoquinol methylase. In the 2A chromosome, QTLs were detected for traits ASS as well as PES between the markers *AX-94961148* (746969247) and *AX-94399039* (715304564). In this large 31.6 Mb long region, 549 genes were predicted. Some promising candidate genes were identified in the interval *viz.*, Carboxypeptidase, Protein phosphatase 2C, 3-ketoacyl-CoA synthase, *WAT1*-related protein, Floral homeotic protein *APETALA* 2, molybdate transporter 2 (MOT2), ABC transporter I family member

1, Glutamine synthetase. In the nearby loci a QTL for SSI (PVE = 15.03%) was also detected however in a different marker interval *AX-94585454* (734256903)– *AX-94407698* (67980360). This is a large interval of 666.3 Mb length where 3927 genes were predicted. In the 3D chromosome QTLs were detected for traits ASS and PES between the markers *AX-95098367* (3980242) and *AX-95091443* (415162203). In this large interval of 411Mb, 2904 genes were predicted. For these two intervals candidate gene prediction was not possible due to large no. of genes.

Meiotic analysis of leaf rust resistant and fertile gamma mutants and their F_1 generation

Cytological analyses were performed on three distinct gamma mutants, along with five F_1s of different gamma mutants. In Sel. 2427, most of the PMCs showed normal configuration of 21^{II} with few exceptions where univalents, trivalents, quadrivalents and isochromosomes were found. In the F_1 generation of Sel. 2427 with Agra Local, severe meiotic instability was visible reducing the frequency of PMCs with 21^{II} (Niranjana et al. 2017). In the gamma mutant T300-67-12-1, out of 49 PMCs observed, all of them showed normal configuration of 21^{II}. While in case of F_1(HD2932 X T300-67-12-1), out of 20 PMCs observed, all of them showed normal configuration of 21^{II}. In the mutant named T300-67-113-17, out of 58 PMCs observed, 57 showed normal configuration of 21^{II} and only one deviated, having $20^{II} + 2^{I}$ (univalent) configuration. While in case of F_1(HD2932 X T300-67-113-17), out of 24 PMCs observed, 23 showed normal configuration of 21^{II} and only one deviated, having $20^{II} + 2^{I}$ configuration. In the mutant named T300-67-6-4, out of 13 PMCs observed, all of them showed normal configuration of 21 bivalents. While in case of F_1(HD2932 X T300-67-6-4), out of 15 PMCs observed, 12 showed normal configuration of 21 bivalents and only 3 deviated, showing $20^{II} + 2^{I}$ configuration. In the case of F_1(HD2932 X T300-67-102-11), out of 15 PMCs observed, all of them showed normal configuration of 21^{II}. In the case of F_1(HD2932 X T300-67-64-60), out of 15 PMCs observed, all of them showed normal configuration of 21^{II}. Since the mutants showed normal 21^{II} configuration in almost all the cells (Suppl. Table 4; Suppl. Figure 2), it is an additional evidence of lack of gametocidal gene(s).

Yield parameters of gamma mutants

Yield parameters such as height, number of tillers, number of spikelets per spike and number of seeds per spike of gamma mutants and control genotypes were measured under field conditions (Fig. 4c). Mean height of T250-20-20-16 is the lowest with 91.80 cm which is comparable with popular varieties HD 3086 (96 cm), HD2932 (91.40 cm) and HD 3059 (90.75 cm). The tallest among the mutants was T300-64-51-28 with a mean height of 128.40 cm. Sel. 2427 (86.50 cm) and ET (83.20 cm) has even lower mean heights and ALS2427-302-4 and ALET62-7 has mean heights of 126.20 cm and 110.20 cm respectively. Mean number of tillers is highest for T300-64-51-28 with a value of 11.80 which is higher than HD2932 (8.00), HD3086 (10.60), HD3059 (8.40). Lowest value came out to be 4.20 for T300-67-113-5. T250-20-20-6 has highest mean number of seeds per spike with a value of 50.36 which is equivalent to HD2932 (50.00), HD3086 (47.72) and HD3059 (50.20). Sel. 2427 (33.75), ET (27.98), ALS2427-302-4

(26.33) and ALET62-7 (22.06) has even less values. Mutant T300-67-113-5 has the lowest value of 23.37. In the case of number of spikelets per spike, the highest mean value recorded is 19.72 in mutant T250-20-20-6 and lowest for mutant T250-20-20-16 with a mean value of 13.49. HD2932, HD3086, HD3059, Sel. 2427, ET, ALS2427-302-4 and ALET62-7 has values 18.30, 16.60, 19.08, 16.85, 13.94, 15.18 and 13.26, respectively. In a nutshell it was observed that the mutants showed superior agronomic performance as compared to original lines Sel. 2427 and ET while they were comparable to popular varieties. However, under field conditions only drawback observed was that all the mutants along with Sel. 2427, ET and RILs showed moderate to high susceptibility to yellow rust.

DISCUSSION

Wild relatives of wheat serve as valuable repositories of beneficial genes associated with yield, quality, tolerance to biotic and abiotic stresses (Baum et al. 1992; Sharma 1995; Singh et al. 2019; Niranjana et al. 2022). Among the wild relatives, species belonging to *Aegilops* genus have been widely used as a good source of rust resistance genes (Riley et al. 1968; Dvorak 1977; Prazak 1997; Tomar and Menon 2001; Aghaee-Sarbarzeh et al. 2002; Ozgen et al. 2004; Singh et al. 2004; Bossolini et al. 2006; Chhuneja et al. 2008; Marais et al. 2008, 2010a, c). Till date, out of 83 designated *Lr* genes, ~ 28 alien leaf rust resistance genes have been reported to be transferred to cultivated wheat varieties (McIntosh et al. 2020; Prasad et al. 2020). Transfer of genes from wild relatives to wheat presents a unique set of adversities. Firstly, for development of alien translocation lines, homoeologous pairing of alien and wheat chromosomes is a prerequisite. For induction of homoeologous pairing, *Ph1* gene needs to be manipulated either by use of *Ph1* deletion mutants (*ph1a* and *ph1b*) (Sears 1972, 1981; Koebner and Shepherd 1986; Rey et al. 2015, 2017a) or by ensuring the absence of chromosome 5B (Nulli 5B) (Riley and Chapman 1958; Sears and Okamoto 1958) in the wide crosses (Rani et al. 2020). However, while utilizing *Ae. speltoides* in wide hybridisation, these chromosomal manipulations are not necessary due to the presence of genes inhibiting/suppressing *Ph1* gene in S genome (Chen et al. 1994; Dvorak et al. 2006; Li et al. 2017). Secondly, even after successful transfer of alien beneficial genes there is possibility of yield penalty and linkage drag associated with the gene of interest in alien translocation line. Until now, six *Lr* genes derived from *Ae. speltoides* (*Lr28, Lr35, Lr36, Lr47, Lr51, Lr66*) have been designated. There are many reports explaining the difficulties faced while gene transfer from *Ae. speltoides* (Friebe et al. 1996; Brevis et al. 2008; Niranjana et al. 2017; King et al. 2018) and many successful incidents where these have been overcome (Friebe et al. 2003; Mago et al. 2009, 2013; Niu et al. 2011). One of the major factors causing linkage drag in *Ae. speltoides* derived translocation lines is the gametocidal activity caused by cuckoo chromosomes. Gametocidal activity has been reported in *Aegilops* species with C, S, S^1, S^{sh}, and M^g genomes with varying level of severity (Grewal et al. 2017; Kishii 2019).

In the present investigation, Sel. 2427 and ET were developed by crossing *T. aestivum* cultivar Sonalika with *Ae. speltoides* acc. EC 331772, followed by two back crosses. Sel. 2427 was found to be resistant against leaf rust (*LrS2427*) as well as stem rust (Dhakate 2002; Durgesh et al. 2011). Nevertheless, Sel. 2427 could not be utilized in resistance breeding since it showed gametocidal activity which manifested

as low pollen fertility and poor seed set in F_1 generation (Niranjana et al. 2017). Hence it was hypothesized that while homoeologous pairing, multiple alien introgressions may have happened in Sel. 2427. Molecular cytogenetic techniques like in situ hybridisation are excellent tools while investigating alien introgression lines (Schwarzacher et al. 1992; Houben et al. 2006; Rey et al. 2015; Han et al. 2022). From preliminary genomic dot blot assay results, presence of S genome in Sel. 2427 and ET was confirmed (Suppl Fig. 1). Genomic in situ hybridisation (GISH) was utilized on mitotic cells of Sel. 2427 to localise the S genome chromosomal segments on wheat chromosomes and it was observed that translocations have happened in six telomeric regions, involving four chromosomes. Two translocations have happened on both telomers in one pair of chromosomes and one translocation on one telomere of one pair of chromosomes (Fig. 5). GISH on pollen mother cells (PMCs) of F_1(Agra Local X Sel. 2427) revealed three translocations as expected (Fig. 6). These evidences point to the fact that unintentional co-transfer of gametocidal gene(s) have occurred along with *LrS2427*.

In the current study, we attempted at identification of QTLs associated with gametocidal activity by dissecting into four component traits *viz*., APF, ASS, SSI and PES. QTLs with major phenotypic effect were observed for all traits except APF and these QTLs mostly localised to chromosomes 2A, 2D and 3D. Earlier studies have revealed involvement of 2S (Tsujimoto and Tsunewaki 1983, 1984, 1985, 1988) and 6S (Kota and Dvorak 1988) of *Ae. speltoides* in imparting gametocidal activity. Candidate gene analysis revealed promising gene(s) associated with seed fertility and grain development in the 2DL (3.2 Mb) and 2AS (31.6 Mb) regions. In both regions, molybdate transporter 2 (*MOT2*) was observed which is reported to be highly expressed in the flower especially in ovaries where import of essential nutrients (molybdate) into developing seeds takes place during senescence (Weber et al. 2023).

Another candidate gene glutamine synthetase was observed in both regions. Glutamine synthetase (*GS*) gene is reported to play a major role in the early stage of seed germination and grain filling in several species like rice (Yabuki et al. 2017; Fujita et al. 2022), barley (Goodall et al. 2013). *GS* is up-regulated during both stages and knockout mutants exhibited low seed yield due to fewer number of siliques, less number of seeds per silique and low dry weight per seed in Arabidopsis (Guan et al. 2015). Another gene carboxypeptidase (*CP*) was predicted in the 2AS region having function in the brassinosteroid (BR) signalling pathway. Overexpression of a gene encoding serine carboxypeptidase (*ECS1-Extra Carpels and Seeds*) increases the number of carpels and seeds per fruit (silique) in Arabidopsis, hence are implicated in reproductive development or produce an increased carpel and seed numbers in any plant species (Wen et al. 2012). In rice, serine carboxypeptidase gene (*SCP46*) is dominantly expressed in developing seeds, particularly in embryo, endosperm and aleurone layers, and its knock-down resulted in smaller grains indicating that it is a master regulator of grain filling (Li et al. 2016). Protein phosphatase 2C (*PP2C*) is another relevant gene in the 2AS interval is reported as downstream factor of cytoplasmic male sterility (CMS) in rice *DCW11* and its knockdown caused a major loss of seed-set fertility, without visible defect in pollen development (Fujii and Toriyama 2008). Another candidate gene in the regions is floral homeotic protein *APETALA* 2, which contributes stochastically in the signalling pathway of flower development (Sharma et al. 2020). Mutation in the *AP2* showed increases in seed size and seed mass

loss-of-function of *BnAP2* gene showed defects in shape, structure, development, and size of seeds in *Brassica napus* (Yan et al. 2012). Even though these genes have been identified to be associated with the manifestation of gametocidal activity in seeds, how these genes act differentially in individuals with heterozygous (F_1 generation) and homozygous (parental generation) *Gc* genes need to be further evaluated to ascertain their role.

In order to ensure the utilization of *LrS2427* in breeding programmes, the unholy association between leaf rust resistance and gametocidal gene(s) need to be broken. Mutagenesis is one of the candidate approaches for getting rid of gametocidal genes (Niranjana 2017). EMS (Ethyl methane sulfonate) induced mutagenesis was successfully utilized to knock out the *Gc2* gene transferred as part of wheat – *Ae. sharonensis* T4B-4Ssh#1 translocation chromosome restoring spike fertility (Friebe et al. 2003). However, in our case EMS mutagenesis could not break the association. Allosyndetic pairing was also successfully utilized to eliminate gametocidal genes (Marais et al. 2010b). Based on the hypothesis that mutation can knock out the gametocidal gene(s), gamma ray mutagenesis was considered as a pre-breeding tool in the present investigation. Since the gametocidal phenotype is expressed in the F_1 generation, the crossed seeds (Agra Local X Sel. 2427) were utilized for mutagenesis instead of Sel. 2427. This helped in identifying mutants (M_0F_1) showing better seed set as compared to control F_1 plants. In the further generations, screening was undertaken for both leaf rust resistance and fertility traits. At M_5F_6 generation, 33 gamma mutants *viz.*, T250-20-12-2, T250-20-12-4, T250-20-20-6, T250-20-20-16, T250-20-44-3, T300-64-51-1, T300-64-51-10, T300-64-51-13, T300-64-51-14, T300-64-51-26, T300-64-51-28, T300-64-70-7, T300-67-6-4, T300-67-6-6, T300-67-22-1, T300-67-64-4, T300-67-64-60, T300-67-64-64, T300-67-64-67, T300-67-93-1, T300-67-93-3, T300-67-101-4, T300-67-102-11, T300-67-102-20, T300-67-113-1, T300-67-113-2-1, T300-67-113-3, T300-67-113-4-1, T300-67-113-5, T300-67-113-17, T300-67-116-12, T300-67-116-12-1, T300-67-116-14 were identified as best candidates. These mutants did not show gametocidal activity in the F_1 generation as evident from the fertility data and meiotic analysis. The gametocidal activity observed in the RILs *viz.*, ALS2427-138-8, ALS2427-302-4, ALS2427-319-3 which are at F_5 generation shows the stubbornness of gametocidal chromosomes of *Ae. speltoides* which is well documented in the literature (King et al. 2018). While the F_1 plants of gamma mutants fared better or on par with the parent mutant on fertility. These gamma mutants are excellent donor genetic stocks for utilisation of *LrS2427* gene in wheat improvement. Study on yield parameters of these mutants revealed that they are agronomically superior than their donor lines Sel. 2427 and ET while being comparable to popular varieties. With their role as donors of the leaf rust resistance gene *LrS2427*, these mutants represent valuable assets in rust resistance focused wheat breeding programs.

Declarations

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Author contributions

Niranjana Murukan and Vinod conceived and designed the experiments; Niranjana Murukan, Shivmangal Singh Tomar, Bhupinder Singh, Shailendra Kumar Jha, Niharika Mallick, Sherry Rachel Jacob, Raghunandan K, Kumble Vinod Prabhu, Vinod were involved in the development of plant materials; Niranjana Murukan, Navpreet Kaur Sekhon, Ragini R, Shailendra Kumar Jha did screening for leaf rust resistance; Ragini R, Niranjana Murukan, Navpreet Kaur Sekhon, Prachi Yadav, Niharika Mallick performed the molecular cytogenetics and meiotic experiments; Ragini R, Chetna Chugh, Niranjana Murukan conducted field experiments and SNP genotyping. Niranjana Murukan, Gitanjali Tandon, Mir Asif Iquebal, Aakriti Verma, Priyanka Agarwal analysed the data and prepared figures; Niranjana Murukan and Ragini R wrote the paper. All authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Data Availability

The data generated and analysed in the current study are available from the corresponding author on reasonable request.

Additional information

Supplementary information

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Figures

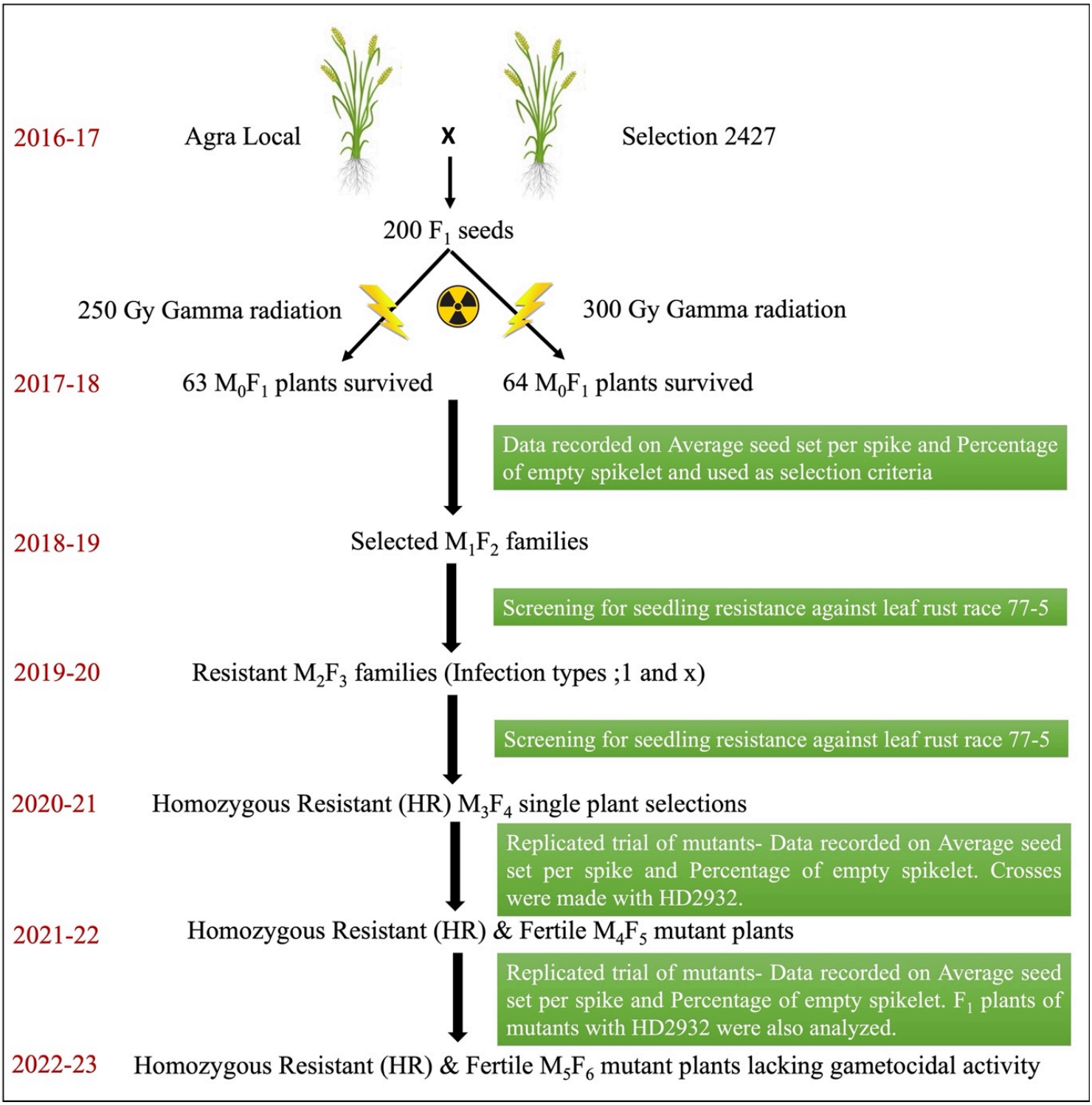


Figure 1

Schematic representation of elimination of gametocidal gene(s) in Sel. 2427 by gamma ray mutagenesis

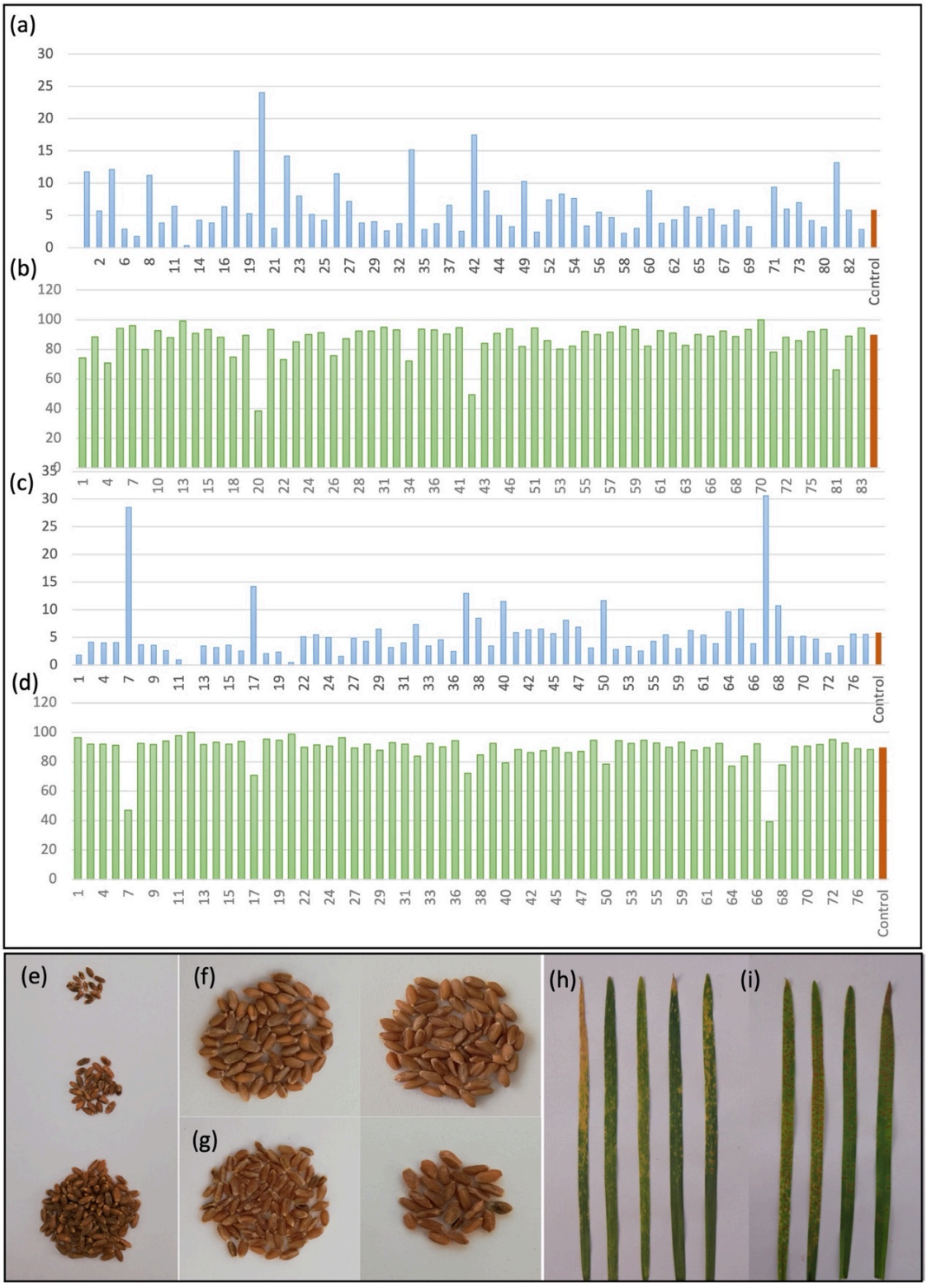


Figure 2

Effect of gamma ray mutagenesis in F_1 (Agra Local X Sel. 2427) plants (a) Improvement in ASS and (b) PES by 250 Gy radiation (c) Improvement in ASS and (d) PES by 300 Gy radiation (e) Seeds harvested from F_1 (Agra Local X Sel. 2427) can be classified into bold, partially shrivelling and completely shrivelling; while seeds harvested from some irradiated F_1 plants showed (f) no shrivelling and (g) some

shrivelling. Single plant selections were made in M_2F_3 families based on seedling resistance to leaf rust race 77-5 and plants with (h) high resistance were selected whereas (i) susceptible plants were discarded.

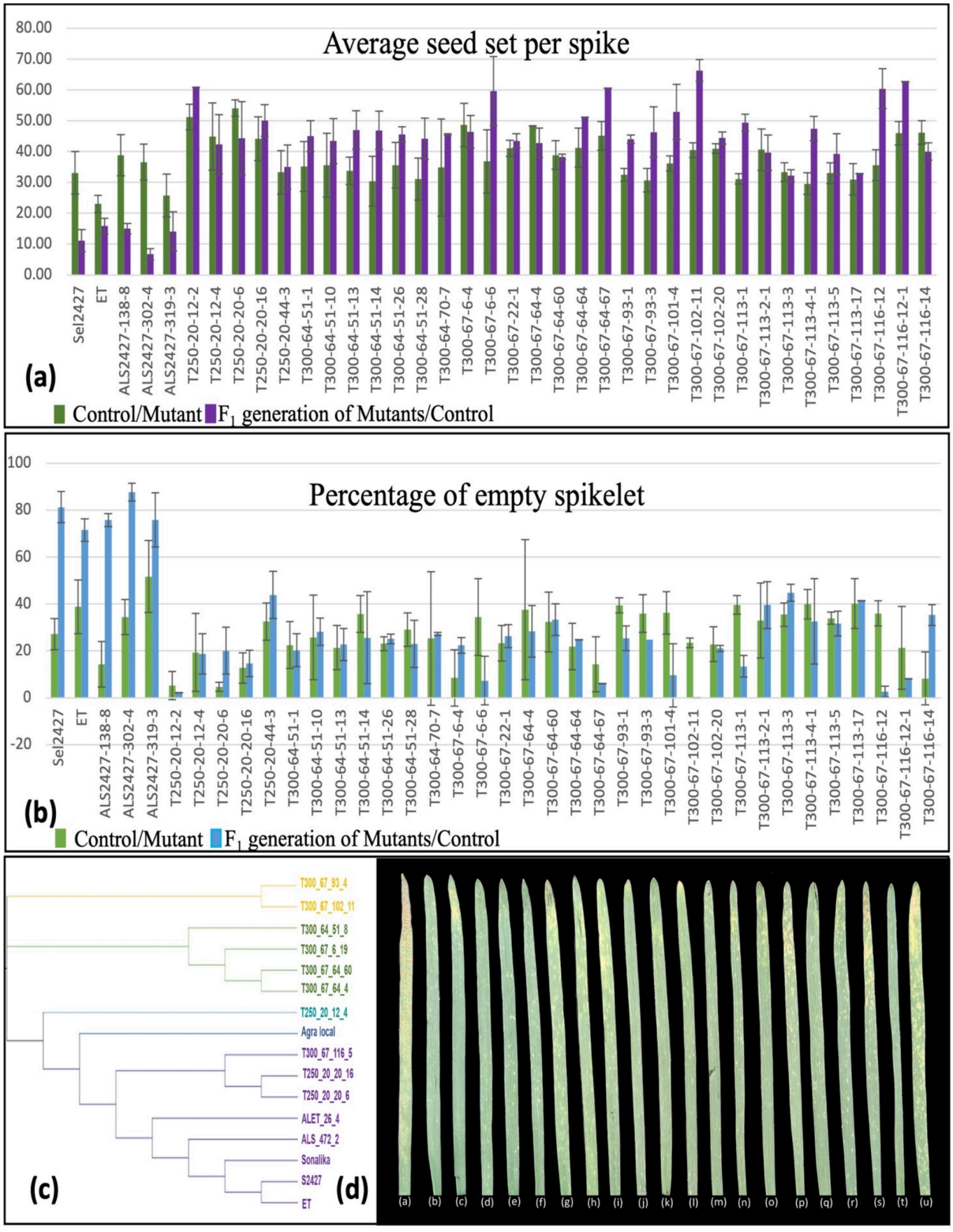


Figure 3

Comparison of (a) average seed set per spike and (b) percentage of empty spikelet in mutants and their corresponding F_1 generation. Calculations for mutants are done on replicated data basis and

calculations for F_1 generation are done on individual plant basis. (c) Dendrogram based on SNP molecular data of gamma mutants and donor lines displaying five clusters (d) Seedling disease reaction of mutants along with susceptible check against leaf rust race 77-5

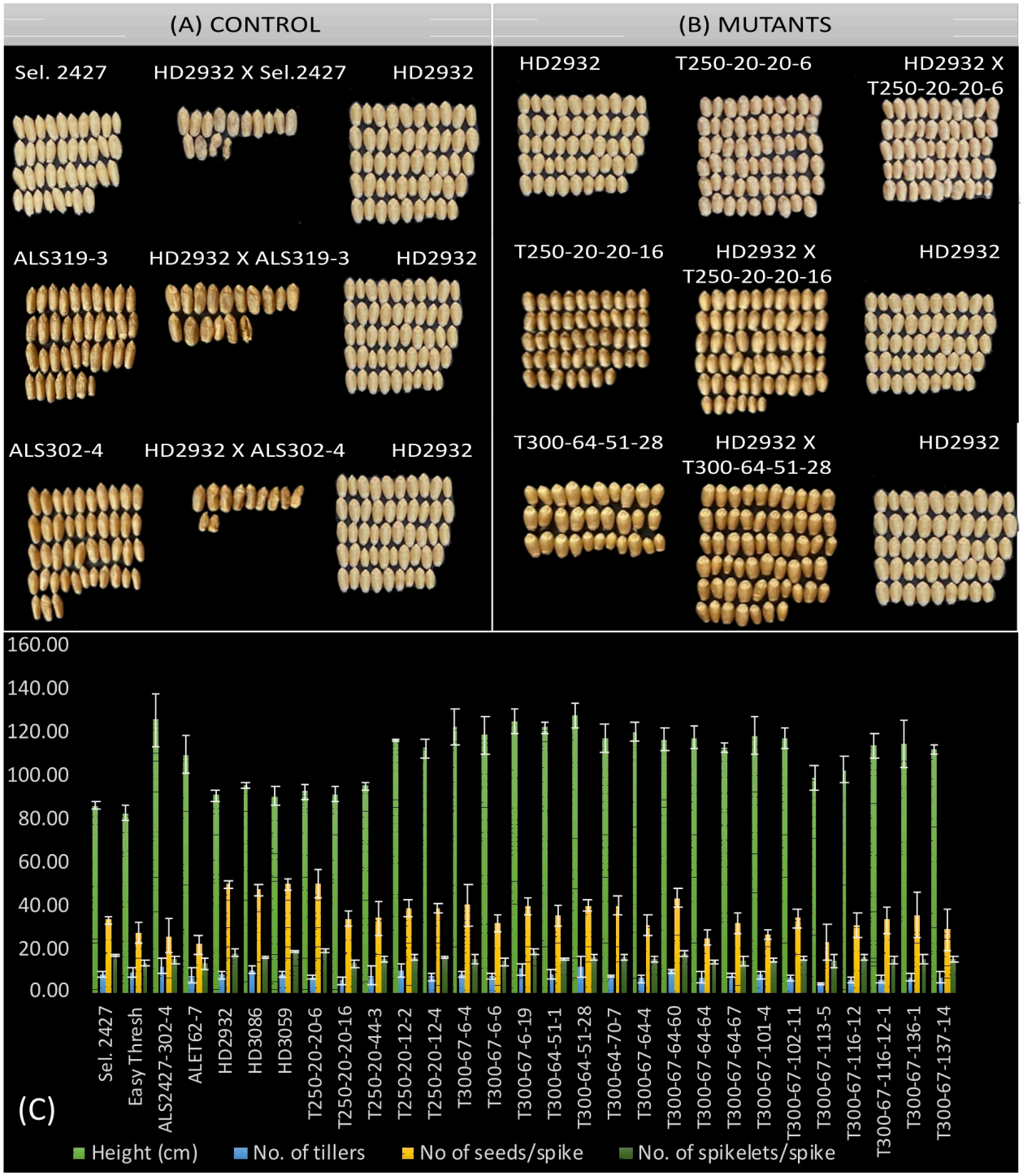


Figure 4

(A & B) Visual comparison of improvement of seed characteristics in mutants and F_1 generation as opposed to Sel. 2427 and control plants (C) Graph showing comparison of yield parameters in mutants

and control genotypes

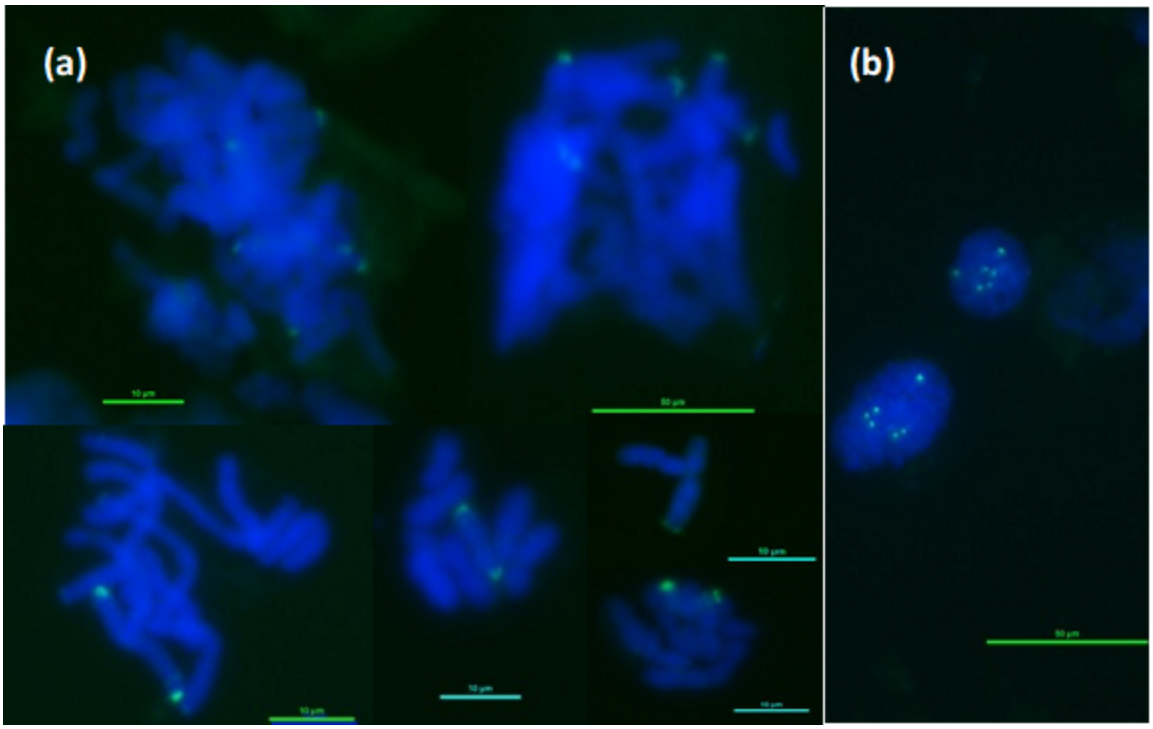


Figure 5

GISH images of Sel. 2427 root cells at (a) 100x and (b) 40x magnification

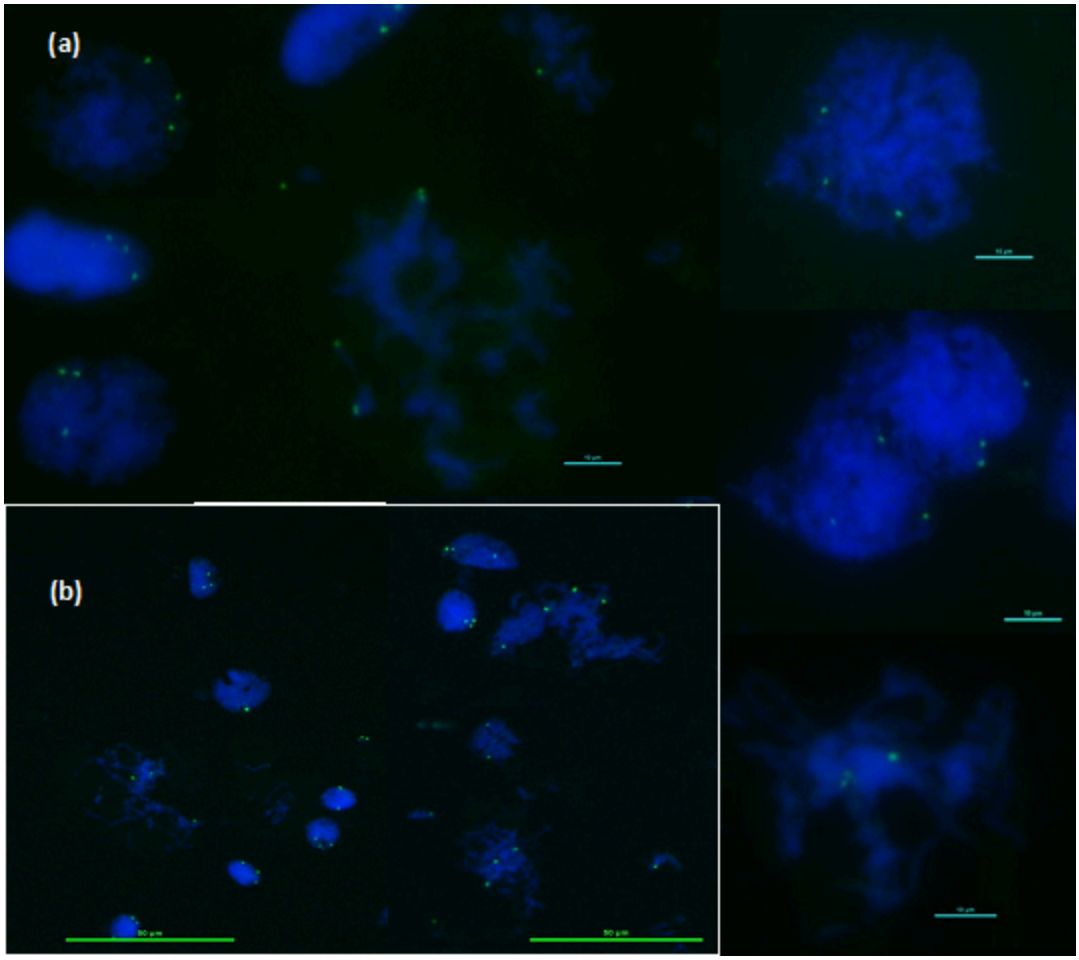


Figure 6

GISH images of PMCs in anthers of F_1(Agra Local X Sel. 2427) in (a) 100x and (b) 40x magnification

Supplementary Files

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