***Original Research Article***

**MARKER ASSISTED BREEDING OF RESISTANCE TO *FUSARIUM* WILT AND PHENOTYPIC EVALUATION OF CHICKPEA BACKCROSS LINES**

**ABSTRACT**

*Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *ciceris* causes extensive damage to chickpea (*Cicer arientinum* L.) in the central part of India, and pathogen race 1 (*Foc* 1) causes severe yield losses. We initiated marker-assisted backcrossing (MABC) using desi landrace, WR 315 as a donor to introgress resistance to race 1 (*Foc* 1) in PKV Kabuli-4, another popular kabuli cultivar of chickpea, which is extra bold seeded, semi-erect, with broad leaves, moderately resistant to wilt, dry rot, and Botrytis gray mildew (BGM), extensively cultivated in the Vidarbha region of Maharashtra. To confirm introgression of resistance for this race, foreground selection was undertaken using three SSR markers (TA59, TA110, and TA194). Based on foreground selection, 28 plants were found heterozygous in BC1F3 for the target alleles. The identified plants were used for the phenotypic evaluation of different agronomic traits. The BC1F3-22 plant had a higher height (75 cm) in comparison to the recurrent parent (48 cm), and the average of all BC1F3 lines showed higher values than the recurrent parent PKV Kabuli-4, i.e., 54.92 cm. The lowest days to 50% flowering were observed in plant BC1F3-21 (49 days), which is less than the recurrent parent. The highest number of primary branches was observed for plant BC1F3-25 (9), which is more than the recurrent (2) and donor parent (7). The days required for the physiological maturity of the BC1F3-12 plant were low compared to both parents. The highest number of pods per plant was recorded on BC1F3-28 (176), which is more than the donor and recurrent parent. The seed weight of all the plants is higher than the donor parents. The grain yield was observed in all plants, which is higher than the donor parent. The utilization of MABC is the most effective and environmentally friendly approach for introgressing complex traits into popular cultivars. There is a need for the deployment of marker-assisted breeding in difficult-to-phenotypically-select traits.

*Keywords: Chickpea, Fusarium wilt, MABC, PKV Kabuli-4, FGS, SSR markers*

**1. INTRODUCTION**

Chickpea (*Cicer arietinum* L.) is a rich source of nutrition and is ranked second amongst food legumes after common bean (Bharadwaj *et al*., 2010). It is a self-pollinated diploid crop with a genome size of 740 Mbp (Varshney *et al*., 2013), 2n = 2x = 16, and grown in nearly 57 countries worldwide (Merga & Haji, 2019). Chickpeas have high nitrogen content, due to their ability to fix atmospheric nitrogen (N) through biological nitrogen fixation, and improve soil fertility, particularly in drylands. Global production has increased significantly in the 21st century, reaching approximately 17 million tons in 2021 (FAOstat, 2023). India is the world’s largest producer of chickpeas, accounting for about 71.2% of global production (FAOstat, 2023). Other chickpea-producing countries are Pakistan with 6.3%, Turkey with 5.2%, Australia with 4.5%, Ethiopia with 3.4%, Myanmar with 2.5%, and Iran with 2.0%. Canada and the United States are relatively minor producers, accounting for only 1.3%-1.5% of global production (FAOstat, 2023). In India, chickpea is cultivated on 9.94 million hectares, with a yield of 11.53 million tons and a productivity of 1160 Kg/ha. The cost is 3527, and the minimum support price (MSP) for chickpea during 2024-25 is 5650 Rs per quintal, with a % return over the cost of 60% (Annual report 2024-25, Department of Agriculture & Framers Welfare, Ministry of Agriculture & Farmers Welfare, Government of India). The major chickpea-producing states in India are Madhya Pradesh, Maharashtra, Rajasthan, Andhra Pradesh, Uttar Pradesh, and Karnataka, which share over 95 percent area in India. In both area and production, chickpea dominates among all pulses in India. Madhya Pradesh leads in chickpea production with a total share of 40.93 %, followed by Maharashtra with a total share of 15.84 % in chickpea production.

Chickpea is a rich source of carbohydrates, essential amino acids, protein, dietary fiber, calcium, iron, and phosphorus (Bampidis and Cristodoulou, 2011). Per 100 grams of chickpea seeds, they have 18.7-23.6 g of protein, 3.7-6.5 g of fat, 39.6-62.6 g of carbohydrates, 3.8-25.2 g of fiber, and 2.7-3.7 g of ash (Mathew *et al.*, 2022). Chickpea is grown on a large scale, but its productivity and production are reduced due to a variety of biotic and abiotic factors, including *Fusarium* wilt, *Ascochyta* blight, insects, nematodes, parasitic weeds, drought, salinity, waterlogging, high temperatures, and chilling. Two fungal diseases, *Ascochyta* blight and *Fusarium* wilt, an economically significant diseases of chickpea (Jendoubi *et al*., 2017). Among the biotic stresses, *Fusarium* wilt is the major disease limiting chickpea productivity in India. Chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceris* was first described by Padwick in India (Padwick, 1940) and reported by Nene in 1980. It is a serious problem in India, Iran, Pakistan, Nepal, Burma, Spain, and Tunisia. In India, it is estimated to cause a 10 per cent annual yield loss (Singh and Dahiya, 1973). The emergence of *Fusarium* wilt (FW) as a devastating root disease of chickpea in central and southern India has been leading to 100% yield losses under favourable conditions (Jendoubi *et al*., 2017). There are eight races of *Fusarium* (race 0, 1A, 1B/C, 2, 3, 4, 5, and 6) which are identified by different disease reactions on a set of chickpea cultivars (Haware and Nene, 1982). Race 1 is widespread in central and peninsular India, and race 2 is in northern India. *Fusarium* wilt is a seed and soil-borne disease. The pathogen can survive in the soil for more than six years even in the absence of chickpea and causes 10%-15% (Biswas and Ali, 2017) of yield losses annually. The disease can occur at all stages of plant growth, with a higher incidence during the flowering and pod stages.

Different disease management methods have been used to control *Fusarium* wilt, such as adjusting the time of sowing and depth, biological control, use of pathogen-free planting material, avoiding sowing into high-risk soils, reduction or elimination of the inoculum in the soil, choice of cropping practices, and chemical control strategies. However, these ineffective management strategies do not control the 100% losses. The most widely accepted and efficient method is to develop and use wilt-resistant genotypes, which is the most effective and eco-friendly method of managing the disease (Sharma and Muehlbauer, 2005; Sabbavarapu *et al*., 2013). To address such a problem, molecular breeding strategies have been deployed in several crop species (Kulwal *et al*., 2011). Marker-assisted backcrossing (MABC) aims to convert targeted lines for one or two traits without disturbing and retaining all other native traits of the target cultivar (Varshney *et al*., 2009). By using different marker genotyping platforms and molecular mapping approaches, significant associations have been identified between markers and the *Foc* resistance gene (Winter *et al*., 2000; Sharma and Muehlbauer, 2007; Jingade and Ravikumar, 2015; Li *et al*., 2015; Mannur *et al*., 2019). Marker-assisted selection based on the molecular markers tightly linked to the wilt resistance trait can be used to screen many chickpea breeding lines/cultivars for the presence of *Foc* genes to develop agronomically superior varieties. The use of MABC has effectively enabled the introgression of *Fusarium* wilt resistance traits in chickpea varieties, including Pusa 391, Hashem, Super Annigeri 1, JG 74, Pusa 256, and C 214 (Bharadwaj *et al*., 2022; Hasaneian Khoshro *et al*., 2024; Mannur *et al*., 2019; Pratap *et al*., 2017; Varshney *et al*., 2014).

PKV Kabuli-4, a popular variety that is extensively grown in the central part of India (Maharashtra and Madhya Pradesh), due to its attractive pinkish white seeds, better dal recovery than desi types, with a lesser soaking period and chaff content. It has become susceptible to FW, and its yield has reduced drastically. Thus, in the present investigation, introgression of race 1 (*Foc* 1) was undertaken at Dr. Panjabrao Deshmukh Krishi Vidyapeeth (PDKV), Akola.

**2. MATERIALS AND METHODS**

**2.1 Selection of markers**

Three simple sequence repeat (SSR) markers, i.e., TA59, TA110, and TA194, confirmed to be linked to quantitative trait loci (QTL) for *Fusarium* wilt resistance (Winter *et al*., 2000; Tekeoglu *et al*., 2000), were used to confirm the plants for the target gene of interest through foreground selection (FGS) at Biotechnology Centre, Department of Agricultural Botany, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India.

**2.2 Selection of plant materials and development of backcross population**

The materials used are 76 BC1F3 lines derived from the cross F1 (PKV Kabuli-4 × WR 315) with the recurrent parent, PKV Kabuli-4 (susceptible to *Fusarium* wilt), which is extra bold seeded, semi-erect, Kabuli type, broad leaves, moderately resistant to wilt, dry rot, and Botrytis gray mildew (BGM) was released in Maharashtra. The WR 315 is a desi landrace from Central India and resistant to wilt in all races; a well-proven resistance source was used as a donor parent. The recurrent parent PKV Kabuli-4 was crossed to a donor parent, WR 315, to generate F1 at the Experimental Field, Biotechnology Centre, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India. The hybridity in F1 was checked with polymorphic SSR markers. True F1s were selected for the first generation of backcrossing with the recurrent parents as females, which was maintained throughout the backcrossing. The backcross progenies at BC1F1 were tested for heterozygosity using three markers (TR19, TA59, TA110, and TA194). The BC1F1 plants were selected based on foreground SSR markers. The selected BC1F1 were selfed up to BC1F3 generation and foreground selection (FGS) and evaluated for agronomic traits.

**2.3 Genomic DNA extraction**

High-quality genomic DNA was extracted from the leaves of the BC1F3 population and their parental genotypes using the cetyltrimethylammonium bromide (CTAB) method with slight modifications (Doyle and Doyle, 1987). Firstly, 150mg of leaf samples were collected from 21-day-old seedlings and grind in 1 ml of 3X extraction buffer. While grinding, 40µl β-mercaptoethanol was added, and samples were incubated at 65 °C for 30 min in a water bath. After incubation, samples were centrifuged at 14000 rpm for 10 min at room temperature. Supernatant was collected, and an equal amount of chloroform-isoamyl alcohol was added. Samples were centrifuged at 11000 rpm for 10 min. Supernatant was collected, and again, an equal amount of chloroform-isoamyl alcohol was added. Centrifugation was done at 11000 rpm for 10 min, and the supernatant was collected. Chilled isopropanol was added in double volume, and samples were incubated at -20 °C for 15-20 min. After precipitation, samples were centrifuged at 14000 rpm for 12 min, and pellets were collected, and 250 µl 70% ethanol was added and centrifuged at 11000 rpm for 5 min. Ethanol was discarded, and the pellet was kept air dry for 20 min. Pellets were dissolved in 40 µl TE buffer, and samples were stored at -20°C for further use.

**2.4 DNA quality checking and quantification**

The DNA quality was checked on a 0.8% agarose gel dissolved in 1x TBE (Tris, Boric, EDTA) buffer. The DNA contents prepared contained 2 µl of DNA, and 3 µl of 6x DNA loading dye, and it was checked on 50 ng and 100 ng lambda DNA (1 µl). This was run in gel electrophoresis (BioRad) at 80V for 45 min. The gel was visualized under a gel documentation system (BioRad). The quantity of DNA samples was assessed by Nanophotometer (Implen). Absorbance was recorded at 260 nm and 280 nm. The value between 1.8 and 2.0 (of the ratio A260/280) was further examined for PCR.

**Table 1. List of primers used for foreground selection against BC1F3 progenies**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sr. No.** | **Marker Name** | **Linkage Group** | **Nucleotide Sequence** | **Annealing**  **Temperature (°C)** | **Reference** |
| 1 | TA59 | LG02 | F: ATCTAAAGAGAAATCAAAATTGTCGAA  R:GCAAATGTGAAGCATGTATAGATAAAG | 57.4 | Winter *et al*., 2000 |
| 2 | TA110 | F: ACACTATAGGTATAGGCATTTAGGCAA  R: TTCTTTATAAATATCAGACCGGAAAGA | 58.9 | Tekeoglu *et al*., 2000; |
| 3 | TA194 | F: TTTTTGGCTTATTAGACTGACTT  R: TTGCCATAAAATACAAAATCC | 51.8 | Winter *et al*., 2000 |

**2.5 PCR amplification**

The PCR was performed in a 10 µl reaction volume. The PCR reaction contained 2 µL of 50 ng DNA, 5 µL of PCR master mix, 0.5 µL each of 10 Pmol forward and reverse primers, and 2 µL of nuclease-free water. The SSR marker fragments were amplified in a 96-well PCR machine (Prima–Trio® Thermal Cycler) using a touchdown programme. The PCR programme consisted of initial denaturation at 94 °C for 3 minutes, followed by the first 5 cycles, which consisted of denaturation at 94 °C for 30 seconds, primer annealing at 65 °C, decreasing by 0.5 °C for 45 seconds, and primer extension at 72 °C for 1 minute. This was followed by 25 cycles of the same denaturation, primer annealing, and primer extension with a final extension step performed at 72 °C for 10 minutes.

**2.6** **Resolution of PCR product by 10 per cent polyacrylamide gel electrophoresis**

The quality of PCR products using 5 µl amplified DNA and 2 µl of 6x DNA loading dye was mixed and checked on a 10% polyacrylamide gel against 100 base pairs (bp) lambda of 50 and 100 ng µl-1. The gel was run on 1x TBE buffer at a constant voltage of 120V for 3 hours. Amplified products were then visualized using the silver staining protocol (Tegelström, 1992). Both glass plates of the PAGE assembly were cleaned with 50 % ethanol and were assembled with spacers and sealed with polyethylene tape from the bottom. The gel solution was prepared with the desired polyacrylamide percentage. After the casting of the gel, it was kept for polymerization for 30-40 min in the PAGE assembly. Water-saturated butanol was topped in casting plates to avoid the contact of monomeric solution with air. After the polymerization was inserted, the comb was removed, and the gel was kept for pre-running in 1X TBE buffer. As pre-running was completed, 5 µl of each PCR amplified sample with 2 µl, 6X loading dye reached nearly 1-2 cm above the bottom of the gel the process of electrophoresis was run off. After electrophoresis, the gel plates were carefully removed from the glass plates and transferred to a tray containing double-distilled water and kept for 5 min with gentle shaking. The distilled water in the above tray was replaced with a fixing solution containing 15 ml of methanol and 750 µl of glacial acetic acid and kept for another 5 min with gentle shaking. The silver solution, i.e., the staining solution, was poured into the tray. The silver solution was prepared by dissolving 0.3 g AgNO3 powder in 150 ml of 10% methanol solution with 750 µl of glacial acetic acid. The solution was kept for 5 min with gentle shaking. The silver solution was removed from the tray, and the gel was rinsed for a while in distilled water. The gel was transferred to a developing solution (prepared by dissolving 9 g NaOH pellets in 300 ml distilled water with 900 µl of formaldehyde. The solution in the tray was shaken gently for 5-10 min, allowing the DNA amplicons to form. The gel was placed on the platform of the Gel Documentation (Eppendorf) system and photographed under EPI white light.

**2.7 Gel Scoring for the foreground selection of BC1F3 population**

Gel scoring was performed manually based on the differential separation of amplicons on 10% PAGE. Using Gene Mapper software version 6.0, the allele of the female parent was always scored as “A” irrespective of the size of the amplicon. Similarly, the allele of the male parent was always scored as “B”, and the genotypes having alleles from both the parents were designated as “H”, and missing data were scored as “-”. Therefore, the allele scoring was carried out as follows –

‘A’ – Allele of female parent (PKV Kabuli-4)

‘B’ – Allele of the male parent (WR 315)

‘H’ – Heterozygous (presence of both parental alleles)

‘-’– Missing data (failed amplification)

The sizes of the bands were estimated by comparing them with a 100 bp standard marker along with both parents. The graphical representation of the foreground selection was done using the graphical genotypes software version GGT 2.0. The software GGT: Graphical Geno Typing V 2.0 is a software package that assists in the graphical representation of molecular marker data, which can assist in the process of selection and evaluation of plant material. This is integrated software for the representation of genotyping data.

**2.8 Phenotypic evaluation of the BC1F3 population**

Phenotypic evaluation of the BC1F3 population and its parents was carried out at the Experimental Field, Biotechnology Centre, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India. The recurrent parent PKV Kabuli-4, donor parent WR 315, and BC1F3 seeds were sown in single-row lengths of 3 m with a spacing of 45 cm x 15 cm. Standard agronomic practices were implemented to promote the growth and development of the crop. The height of each plant was measured from the base of the plant to the tip of the main shoot from five randomly selected plants, and the average plant height was calculated in centimeters. Days to 50% flowering were measured by counting the days from the date of sowing to the opening of flowers in 50% of plants on each plant. The total number of primary branches was measured from the individual plant at the time of maturity of the plant. The number of days required from sowing to full maturity in each plant was recorded as days to maturity. The total number of pods per plant was counted at the time of maturity or harvesting. For the measurement of 100 seed weight (g), 100 seeds were taken, which were produced from the backcross generation, and seed weight was recorded in grams. In cases where the seed number was less than 100, it was calculated from the weight of the available number of seeds and converted to 100 seed weight. The total seed from each plant was weighed and recorded in grams (g) after threshing the dried pods as a grain yield per plant (g).

**3. RESULTS AND DISCUSSION**

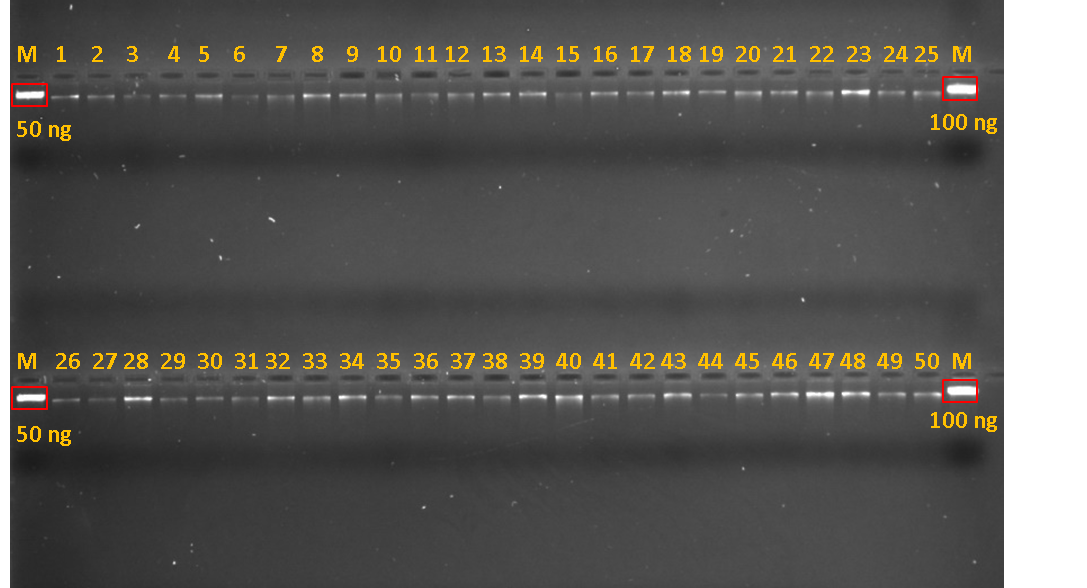
**3.1 Foreground selection of the BC1F3 population**

The isolated genomic DNA quality was checked on a 0.8% agarose gel along with 50 ng lambda DNA (**Figure 1**). After quality checking, DNA was quantified on a nanophotometer at 260/280 nm with nucleic acid factor 50, and the concentration of DNA was recorded. The value between 1.8 – 2.0 (of ratio A260/A280) was taken further for PCR. Nanodrop reading of the isolated genomic DNA of BC1F3 lines used for the PCR amplification is given in **Appendix 1**. The DNA was normalized up to 50 ng/µl with nuclease-free water for PCR amplification

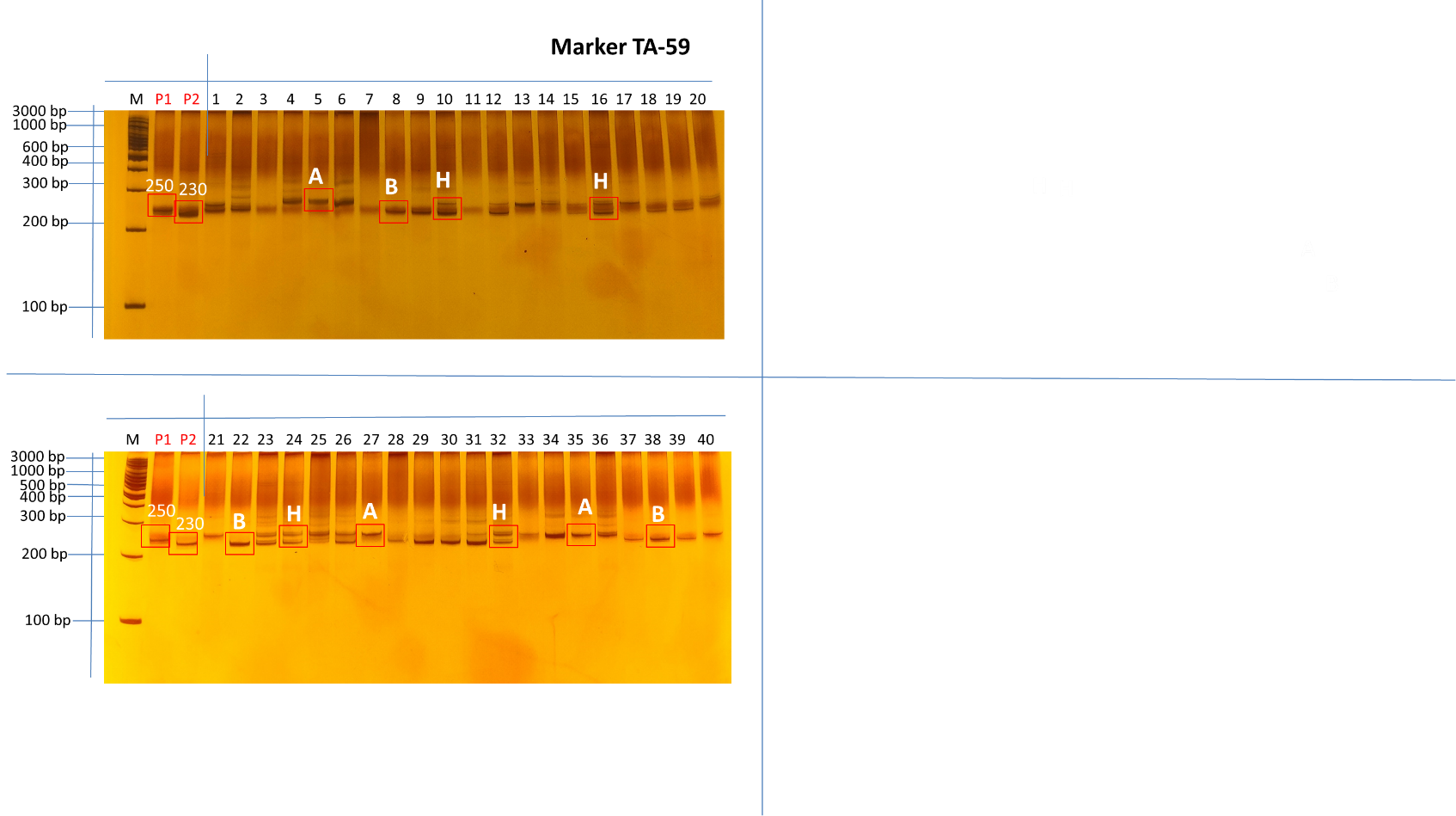
Plant-derived from the crossing process were checked for the presence of desirable alleles using target *Foc*-1 linked markers, namely, TA59, TA110, and TA194 from LG-02, which are reported to be linked with *Fusarium* wilt races. Foreground selection for the *Foc*-1 loci was affected using tightly linked molecular markers, TA59, TA110, and TA194. Out of 76, the marker TA59 showed 28, the marker TA110 showed 22, and the marker TA194 showed 24 heterozygous plants. The electrophoretic image of foreground selection is shown in **Figure 2.** The tested backcross displayed that these plants were true to the target location. The 76 BC1F3 plants were tested by linked foreground markers, and 28 heterozygous plants were used for the second round of backcrossing with the recurrent parent. Scoring of foreground selection using linked markers in the BC1F3 population was given in **Appendix 2**. For further crossing procedure, the common positive plants for the target alleles shown by the linked markers TA-59, TA-110, and TA-194 were used as the female parent, and the recurrent parent PKV Kabuli-4 was used as the male parent. The scoring of foreground selection using linked markers in BC1F3 plants is given in **Table 2**. The graphical representation of the foreground selection of the BC1F3 population was done using the graphical genotypes software version GGT 2.0 (**Figure 3**).

**Table 2. Scoring of foreground selection using linked markers in the BC1F3 population**

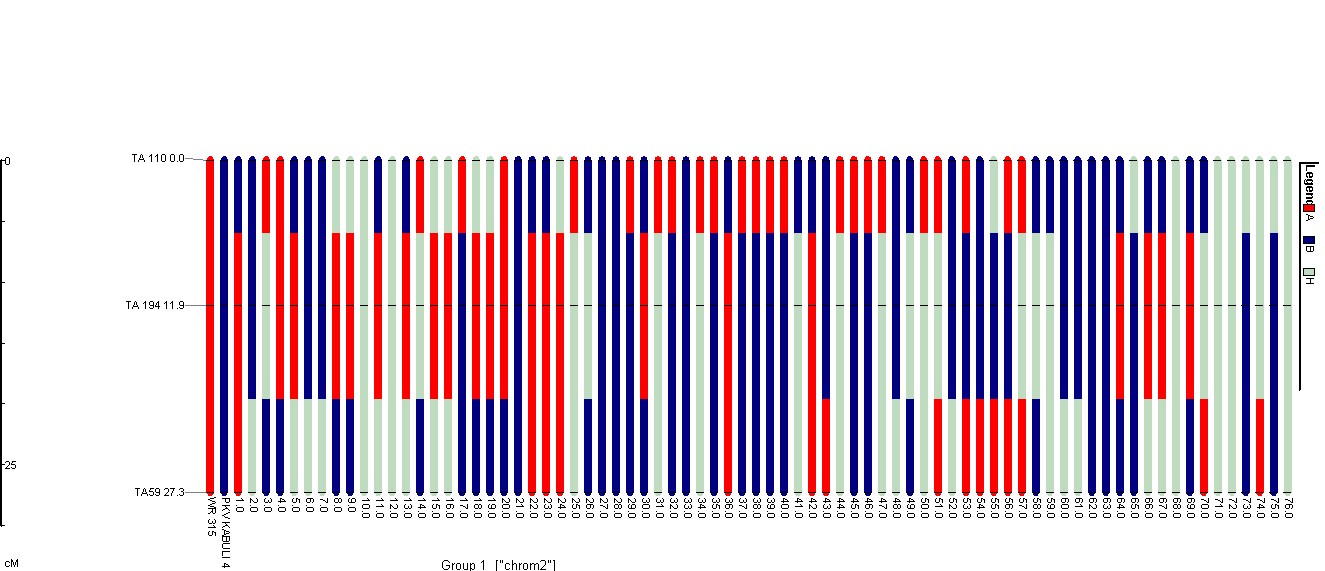
|  |  |  |  |
| --- | --- | --- | --- |
| **Plant Name** | **Cross (F1** × **PKV Kabuli-4)** | | |
| **Marker** | | |
| **Marker TA59** | **Marker TA110** | **Marker TA194** |
| **P1 (WR 315)** | 250 bp | 230 bp | 240 bp |
| **P2 (PKV Kabuli-4)** | 230 bp | 220 bp | 230 bp |
| **Total BC1F3 plants screened** | 76 | 76 | 76 |
| **Total Heterozygous Plants** | **28** | **22** | **24** |

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**Figure 1. Integrity of genomic DNA isolated from the BC1F3 population**

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**Figure 2. Foreground selection of the BC1F3 population derived from the selfing of the BC1F1 population through PCR using the TA59 marker. P1 indicates donor parent WR 315; P2 indicates recurrent parent PKV Kabuli-4; A shows the P1 type alleles; B indicates the P2 type alleles; H indicates the heterozygotes; the number on top of each gel represents BC1F3 progenies.**



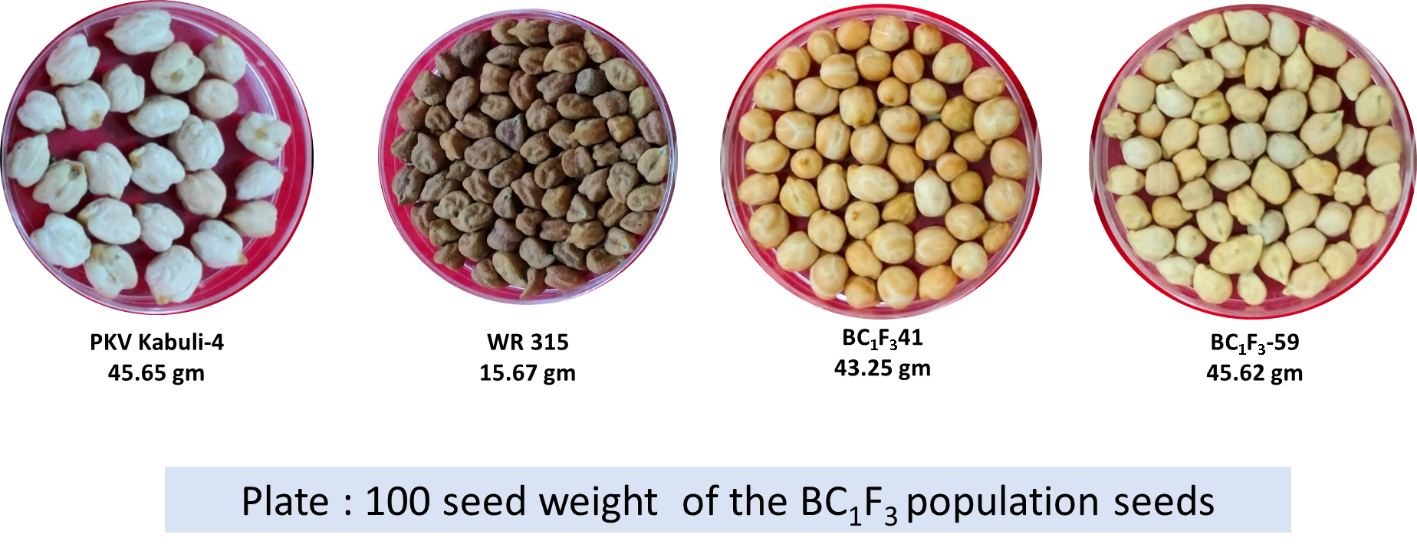
**Figure 3. Graphical representation of foreground selection of the BC1F3 population using SSR markers**

**3.2 Performance of chickpea BC1F3 population derived from the selfing of BC1F1 for different morphological traits**

Foreground selection was coupled with stringent phenotypic selection for agronomic and yield-attributing traits to accelerate the recovery of the recurrent parent genome. BC1F3 plants were selected for morphological characters like plant height (cm), days to 50% flowering, number of primary branches, days to maturity, pods per plant, 100 seed weight (g), and seed yield per plant (g). The morphological observations recorded during the Rabi 2022-2023 and screening of the BC1F3 population for different morphological traits are given in **Table 3**.

Plant height ranges from 21 cm (BC1F3-9) to 75 cm (BC1F3-22) as compared to the mean values of parents, i.e., 48 cm and 42 cm for recurrent and donor parents, respectively. The highest plant height (cm) was observed in plant BC1F3-22, i.e., 75 cm, and the average (54.92 cm) of all BC1F3 lines showed higher values than the recurrent parent PKV Kabuli-4. Days required for 50% flowering of each plant were recorded, and the range was 49 days for (BC1F3-21) to 68 days (BC1F3-16) as compared to the mean value of 50 and 58 for (PKV Kabuli-4) and for (WR-315) parents, respectively. The lowest days to 50% flowering were observed in plant BC1F3-21, i.e., 49, which is less than the recurrent parent, i.e., 50, and the average is 57.07. Several primary branches for BC1F3 progenies were recorded from 2 to 9, as compared to 3 and 7 mean values of recurrent parent PKV Kabuli-4 and donor parent WR 315, respectively. The highest number of primary branches was observed for plant BC1F3-25, i.e., 9, and the average is 5. The days required for the physiological maturity of each plant were recorded and ranged between 101 days after sowing (BC1F3-12) to 121 DAS (BC1F3-14) as compared to their parent, i.e., 115 and 109 for (PKV Kabuli-4) and (WR-315), respectively. The average value for days to maturity is 111.46.

The number of pods per plant was recorded and ranged from 60 for the plant (BC1F3-5) to 176 for (BC1F3-28), as compared to 60 (PKV Kabuli-4) and 75 (WR-315) parents. The highest number of pods per plant was observed in plant BC1F3-28, i.e., 176, and the average is 86.25. The seed weight in grams was recorded and ranged from 36.5 g for (BC1F3-10) to 44.9 g for (BC1F3-2), as compared to the 45 g for (PKV Kabuli-4) and 15 g for (WR-315) of the two crossed parents. The 100 seed weight of BC1F3 progenies along with the donor parent (WR 315) and recurrent parent (PKV Kabuli-4) is shown in **Figure 4**. The total seed from each plant was weighed and recorded in grams (g) after threshing the dried pods. The grain yield was observed from 37.99 g for the plants (BC1F3-12, BC1F3-14, and BC1F3-27) to 46.61 g for BC1F3-11 and BC1F3-15, as compared to 35.32 g (PKV Kabuli-4) and 47.55 g (WR-315) parents. The average grain yield per plant is 41.98 g.

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**Figure 4. 100 seed weight of recipient parent, donor parent, and promising BC1F3 lines**

*Fusarium* wilt is the most damaging disease of chickpeas, resulting in significant production losses. Conventional disease-resistance breeding procedures are laborious and time-consuming. However, MABC applying Foreground selection (FGS) using QTL-linked markers is an environment-independent, precise, and quick approach for the development of cultivars of the trait of interest (Varshney et al., 2010). This work demonstrates the effective introgression of *Fusarium* wilt (FW) resistance into the PKV kabuli-4 genetic background.

Quantitative trait loci (QTL) mapping identified resistance loci with flanking molecular markers for resistance to the *Foc* 1 locus for race 1 (Gowda *et al*., 2009). Two QTLs (FW-Q-APR-6-1 and FW-Q-APR-6-2) for FW for race 1 were identified, accounting for 10.4 to 18.8% of phenotypic variance (Sabbavarapu *et al*., 2013). New QTLs for race 1 were identified (Patil *et al*., 2014; Jingade & Ravikumar, 2015; Garg *et al*., 2018). As previously stated, the *Foc* 1 gene, which confers resistance to FW race 1, is situated in linkage group 2 (LG2) and was targeted for introgression into the recurrent parent PKV Kabuli-4. To choose positive plants for crossing or selfing, foreground selection (FGS) using QTL-linked markers was done.

Marker-assisted backcrossing (MABC) has been successfully employed recently to introgress the “QTL-hotspot” for root traits and other drought tolerance traits in JG11, a leading chickpea variety in India, from the donor parent ICC4958. A “QTL-hotspot” containing QTL for several root and drought tolerance traits. Foreground selection was carried out with three SSR markers, namely TAA170, ICCM0249, and STMS11 (Varshney *et al*., 2013). *Fusarium* wilt is a major disease of chickpea, particularly under dry and warm, humid conditions, resulting in significant yield losses. Chemical control of this soil-borne pathogen is ineffective and has negative environmental impacts. Use of disease-resistant cultivars is the most effective and environmentally friendly approach for control of the pathogen. Therefore, a plant breeder would prefer to exercise marker-assisted backcrossing (MABC) for the development of superior cultivars with the desired trait.

Given the above, PKV Kabuli-4 is the most popular variety among the farmers in the Vidarbha region of Maharashtra and has become susceptible to *Fusarium* wilt due to the incidence of the pathogen in farmers' fields. As a result, there has been a significant reduction in production and productivity. Because of this, the present study was undertaken to introgress the *Foc* 1 locus conferring resistance to race 1 of FW (prevalent in the Vidarbha region of Maharashtra) into PKV Kabuli-4 by employing MABC at PDKV Akola. WR 315, a desi landrace from central India, resistant to races like 1A, race 2, race 3, race 4, and race 5, with target loci for resistance to *Foc* 1 (Sharma and Muehlbauer, 2005), was chosen as the donor parent. While the traditional backcrossing and selfing approach was used to advance the generations, foreground selection with linked SSR markers employed the backcross generation to identify true plants for either crossing or selfing.

**Table 3. Phenotypic evaluation of the BC1F3 population derived from the selfing of the BC1F1 population**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sr. No.** | **Name of Plants** | **Plant Height (cm)** | **Days to 50 % Flowering** | **Number of Primary Branches** | **Days to Maturity** | **Pods Per Plant** | **100 Seed**  **Weight (g)** | **Seed Yield Per Plant (g)** |
| 1 | BC1F3-1 | 48 | 50 | 6 | 115 | 93 | 39.5 | 40.52 |
| 2 | BC1F3-2 | 53 | 58 | 8 | 109 | 65 | 44.9 | 38.23 |
| 3 | BC1F3-3 | 57 | 54 | 5 | 110 | 93 | 42.1 | 44.91 |
| 4 | BC1F3-4 | 53 | 51 | 5 | 110 | 97 | 38.6 | 40.47 |
| 5 | BC1F3-5 | 43 | 66 | 2 | 110 | 60 | 41.6 | 43.56 |
| 6 | BC1F3-6 | 58 | 53 | 8 | 108 | 117 | 42.3 | 45.73 |
| 7 | BC1F3-7 | 65 | 62 | 7 | 118 | 76 | 39.2 | 42.63 |
| 8 | BC1F3-8 | 53 | 66 | 4 | 108 | 69 | 40.9 | 44.12 |
| 9 | BC1F3-9 | 31 | 59 | 4 | 110 | 77 | 40.5 | 38.54 |
| 10 | BC1F3-10 | 58 | 50 | 3 | 109 | 84 | 36.5 | 41.97 |
| 11 | BC1F3-11 | 56 | 58 | 4 | 106 | 92 | 41.8 | 46.61 |
| 12 | BC1F3-12 | 55 | 50 | 3 | 101 | 89 | 44.7 | 37.99 |
| 13 | BC1F3-13 | 59 | 60 | 5 | 109 | 64 | 43.5 | 40.38 |
| 14 | BC1F3-14 | 54 | 61 | 5 | 121 | 94 | 42.6 | 37.99 |
| 15 | BC1F3-15 | 49 | 60 | 6 | 119 | 72 | 41.5 | 46.61 |
| 16 | BC1F3-16 | 48 | 68 | 3 | 116 | 82 | 38.9 | 41.97 |
| 17 | BC1F3-17 | 50 | 50 | 4 | 117 | 96 | 43.1 | 38.54 |
| 18 | BC1F3-18 | 62 | 48 | 6 | 107 | 84 | 44.6 | 44.12 |
| 19 | BC1F3-19 | 59 | 53 | 4 | 116 | 63 | 39.6 | 42.63 |
| 20 | BC1F3-20 | 43 | 56 | 4 | 109 | 78 | 42.8 | 45.73 |
| 21 | BC1F3-21 | 55 | 49 | 4 | 107 | 103 | 38.5 | 43.56 |
| 22 | BC1F3-22 | 75 | 60 | 8 | 115 | 83 | 40.8 | 40.47 |
| 23 | BC1F3-23 | 63 | 60 | 3 | 109 | 72 | 43.2 | 44.91 |
| 24 | BC1F3-24 | 59 | 60 | 4 | 110 | 91 | 41.7 | 38.23 |
| 25 | BC1F3-25 | 65 | 60 | 9 | 110 | 108 | 41.9 | 40.52 |
| 26 | BC1F3-26 | 42 | 50 | 6 | 116 | 71 | 40.3 | 44.12 |
| 27 | BC1F3-27 | 63 | 66 | 4 | 108 | 66 | 38.7 | 37.99 |
| 28 | BC1F3-28 | 62 | 60 | 6 | 118 | 176 | 39.8 | 42.63 |
|  | **WR 315** | 42 | 58 | 7 | 109 | 75 | 45.6 | 47.55 |
|  | **PKV Kabuli-4** | 48 | 50 | 3 | 115 | 60 | 15.6 | 35.32 |
|  | **SD** | **8.82307** | **5.90623** | **1.78471** | **4.74913** | **22.77** | **2.09869** | **2.84866** |
|  | **Average** | **54.9286** | **57.0714** | **5** | **111.464** | **86.25** | **41.2179** | **41.9886** |

**4. CONCLUSION**

The BC1F3 population was screened through foreground markers, namely TA59, TA110, and TA194, for the selection of positive plants. The marker TA59 produces a 250 bp size allele in the donor parent WR 315 and a 230 bp allele in the recurrent parent PKV Kabuli-4, while marker TA110 produces a 230 bp size allele in the donor and a 220 bp allele in the recurrent parent. However, the marker TA194 produces the 240 bp size allele in the donor and the 230 bp size allele in the recurrent parent PKV kabuli-4. The BC1F3-22 plant had a higher height in comparison to the recurrent parent, and the average of all BC1F3 lines showed higher values than the recurrent parent, PKV Kabuli-4. The lowest days to 50% flowering were observed in plant BC1F3-21, which is less than the recurrent parent. The highest number of primary branches was observed for plant BC1F3-25, which is more than the recurrent and donor parent. The days required for the physiological maturity of the BC1F3-12 plant were low compared to both parents. The highest number of pods per plant was recorded on BC1F3-28, which is more than the donor and recurrent parent. The seed weight of all the plants is higher than the donor parents. The total seed from each plant was weighed and recorded in grams (g) after threshing the dried pods. The grain yield was observed in all plants, which is higher than the donor parent. The identification of the backcross lines was possible through the utilization of linked QTL markers. This may suggest that the successful introgression of the QTL region in the genetic background of recurrent parents is expected to improve the chickpea yield. Further, lines with improved *Fusarium* wilt resistance could also be identified as donor parents for future breeding programs. From the study, lines identified to be better than parents need to be backcrossed for the recovery of the recurrent parent genome.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Authors hereby declare that no generative AI technologies such as large language models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

**ETHICAL APPROVAL**

This article does not contain any studies with human participants or animals performed by any of the authors.

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**ABBREVIATIONS**

%: Per cent

°C: Degree Celsius

µl: Microliter (S)

BC: Backcross

Bp: Base pairs

Cm: Centimeter

CTAB: Cetyl Trimethyl Ammonium Bromide

DAS: Days After Sowing

EDTA: Ethylenediamine tetraacetic acid

Et al.: Et alia (and others)

FGS: Foreground Selection

Foc: *Fusarium oxysporum* f. sp. *ciceris*

FW: *Fusarium* wilt

G: Gram

LG: Linkage group

MABC: Marker-Assisted Backcrossing

Mbp: Mega base pairs

Min: Minute

ng: Nanogram

nm: Nanometer

PCR: Polymerase Chain Reaction

QTL: Quantitative Trait Loci

RPM: Revolution Per Minute

SD: Standard Deviation

SSR: Simple Sequence Repeat

TBE buffer: Tris hydroxymethyl aminomethane, boric acid, ethylene diamine tetraacetic acid buffer

TE buffer: Tris hydroxymethyl aminomethane, boric acid, ethylene diamine tetraacetic acid buffer

V: Volt

**DEFINITIONS**

Allele: An alternative form of a gene.

Backcross: A cross of a hybrid with one of its parents.

Genotype: The genetic constitution, i.e., genes that make up an organism.

Linkage: The relationship between two or more genes that tend to be inherited together because they are located on the same chromosome.

Linkage group: A group of genes is arranged in a linear order on a chromosome.

Locus: A stretch of DNA at a particular place on a particular chromosome - often used for a 'gene' in a broad sense.

Marker-assisted Breeding: Marker-assisted breeding is a technique that uses DNA markers associated with desirable traits that can be used in plant improvement in breeding programs.

Molecular Breeding: Improvement of crop plants for various economic characters through indirect selection for linked molecular markers.

Selection: It aims at isolating the desirable ones from the mixture of numerous genotypes in the population.

Nucleotides: The building blocks of DNA (and RNA). DNA nucleotides comprise a nitrogenous base, a deoxyribose sugar, and a phosphate group.

PCR: A technique for producing millions of copies of a DNA sequence.

**APPENDIX**

**Appendix 1. Nanodrop reading of the isolated genomic DNA of BC1F3 lines used for molecular analysis**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sr. No.** | **Sample ID** | **Nucleic Acid (ng/µL)** | **A260/A280 Ratio** | **Nucleic Acid Factor** | **Baseline Correction (nm)** |
|  | WR 315 | 2.18 | 2270 | 50 | 340 |
|  | PKV Kabuli-4 | 1.85 | 3492 | 50 | 340 |
| 1 | BC1F3-1 | 1.86 | 3392 | 50 | 340 |
| 2 | BC1F3-2 | 1.61 | 3786 | 50 | 340 |
| 3 | BC1F3-3 | 1.79 | 4255 | 50 | 340 |
| 4 | BC1F3-4 | 1.67 | 4389 | 50 | 340 |
| 5 | BC1F3-5 | 1.98 | 3924 | 50 | 340 |
| 6 | BC1F3-6 | 1.88 | 4125 | 50 | 340 |
| 7 | BC1F3-7 | 1.98 | 3765 | 50 | 340 |
| 8 | BC1F3-8 | 1.75 | 2320 | 50 | 340 |
| 9 | BC1F3-9 | 2.00 | 2880 | 50 | 340 |
| 10 | BC1F3-10 | 1.65 | 3720 | 50 | 340 |
| 11 | BC1F3-11 | 1.75 | 1472 | 50 | 340 |
| 12 | BC1F3-12 | 1.96 | 1595 | 50 | 340 |
| 13 | BC1F3-13 | 1.93 | 3041 | 50 | 340 |
| 14 | BC1F3-14 | 1.84 | 97.63 | 50 | 340 |
| 15 | BC1F3-15 | 1.63 | 1961 | 50 | 340 |
| 16 | BC1F3-16 | 1.81 | 3017 | 50 | 340 |
| 17 | BC1F3-17 | 1.76 | 3613 | 50 | 340 |
| 18 | BC1F3-18 | 2.20 | 702.8 | 50 | 340 |
| 19 | BC1F3-19 | 1.80 | 3968 | 50 | 340 |
| 20 | BC1F3-20 | 1.66 | 4007 | 50 | 340 |
| 21 | BC1F3-21 | 1.90 | 3774 | 50 | 340 |
| 22 | BC1F3-22 | 2.07 | 2211 | 50 | 340 |
| 23 | BC1F3-23 | 1.74 | 2212 | 50 | 340 |
| 24 | BC1F3-24 | 1.77 | 1426 | 50 | 340 |
| 25 | BC1F3-25 | 1.85 | 1226 | 50 | 340 |
| 26 | BC1F3-26 | 1.96 | 3828 | 50 | 340 |
| 27 | BC1F3-27 | 1.94 | 3827 | 50 | 340 |
| 28 | BC1F3-28 | 1.88 | 3743 | 50 | 340 |
| 29 | BC1F3-29 | 1.94 | 3642 | 50 | 340 |
| 30 | BC1F3-30 | 1.89 | 3924 | 50 | 340 |
| 31 | BC1F3-31 | 1.64 | 4433 | 50 | 340 |
| 32 | BC1F3-32 | 1.78 | 4104 | 50 | 340 |
| 33 | BC1F3-33 | 1.72 | 1821 | 50 | 340 |
| 34 | BC1F3-34 | 1.67 | 3476 | 50 | 340 |
| 35 | BC1F3-35 | 1.66 | 1556 | 50 | 340 |
| 36 | BC1F3-36 | 1.77 | 2763 | 50 | 340 |
| 37 | BC1F3-37 | 2.12 | 1877 | 50 | 340 |
| 38 | BC1F3-38 | 2.09 | 1485 | 50 | 340 |
| 39 | BC1F3-39 | 2.00 | 3344 | 50 | 340 |
| 40 | BC1F3-40 | 2.06 | 2383 | 50 | 340 |
| 41 | BC1F3-41 | 1.88 | 3486 | 50 | 340 |
| 42 | BC1F3-42 | 1.96 | 2808 | 50 | 340 |
| 43 | BC1F3-43 | 1.82 | 4018 | 50 | 340 |
| 44 | BC1F3-44 | 1.91 | 3927 | 50 | 340 |
| 45 | BC1F3-45 | 1.86 | 4203 | 50 | 340 |
| 46 | BC1F3-46 | 2.01 | 3154 | 50 | 340 |
| 47 | BC1F3-47 | 1.92 | 3855 | 50 | 340 |
| 48 | BC1F3-48 | 2.02 | 2799 | 50 | 340 |
| 49 | BC1F3-49 | 1.86 | 3496 | 50 | 340 |
| 50 | BC1F3-50 | 1.61 | 3752 | 50 | 340 |
| 51 | BC1F3-51 | 2.05 | 2604 | 50 | 340 |
| 52 | BC1F3-52 | 1.98 | 3556 | 50 | 340 |
| 53 | BC1F3-53 | 2.05 | 2663 | 50 | 340 |
| 54 | BC1F3-54 | 2.04 | 2866 | 50 | 340 |
| 55 | BC1F3-55 | 2.06 | 2117 | 50 | 340 |
| 56 | BC1F3-56 | 2.06 | 2248 | 50 | 340 |
| 57 | BC1F3-57 | 2.11 | 844.1 | 50 | 340 |
| 58 | BC1F3-58 | 1.73 | 3674 | 50 | 340 |
| 59 | BC1F3-59 | 2.08 | 1930 | 50 | 340 |
| 60 | BC1F3-60 | 2.01 | 3118 | 50 | 340 |
| 61 | BC1F3-61 | 1.90 | 3956 | 50 | 340 |
| 62 | BC1F3-62 | 1.64 | 1718 | 50 | 340 |
| 63 | BC1F3-63 | 1.97 | 3114 | 50 | 340 |
| 64 | BC1F3-64 | 1.52 | 4032 | 50 | 340 |
| 65 | BC1F3-65 | 1.82 | 3234 | 50 | 340 |
| 66 | BC1F3-66 | 1.84 | 4391 | 50 | 340 |
| 67 | BC1F3-67 | 1.78 | 4305 | 50 | 340 |
| 68 | BC1F3-68 | 1.93 | 3642 | 50 | 340 |
| 69 | BC1F3-69 | 2.23 | 2911 | 50 | 340 |
| 70 | BC1F3-70 | 2.07 | 1197 | 50 | 340 |
| 71 | BC1F3-71 | 1.75 | 3700 | 50 | 340 |
| 72 | BC1F3-72 | 2.09 | 1962 | 50 | 340 |
| 73 | BC1F3-73 | 1.92 | 3761 | 50 | 340 |
| 74 | BC1F3-74 | 1.64 | 1248 | 50 | 340 |
| 75 | BC1F3-75 | 1.87 | 4054 | 50 | 340 |
| 76 | BC1F3-76 | 1.74 | 4489 | 50 | 340 |

**Appendix 2.** **Scoring of foreground selection using linked markers in the BC1F3 population**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sr. No.** | **Plant Name** | **Marker TA-59** | **Marker-TA110** | **Marker TA-194** |
|  | WR 315 | 240 | 230 | 240 |
|  | PKV Kabuli-4 | 250 | 220 | 230 |
| 1 | BC1F3-1 | 230/250 | 220 | 240 |
| 2 | BC1F3-2 | 250 | 220 | 230 |
| 3 | BC1F3-3 | 230 | 230 | 230/240 |
| 4 | BC1F3-4 | 230/250 | 230 | 240 |
| 5 | BC1F3-5 | 230 | 220 | 240 |
| 6 | BC1F3-6 | 230/250 | 220 | 230 |
| 7 | BC1F3-7 | 230 | 220 | 230 |
| 8 | BC1F3-8 | 230 | 220/230 | 240 |
| 9 | BC1F3-9 | 230 | 220/230 | 240 |
| 10 | BC1F3-10 | 230/250 | 220/230 | 230/240 |
| 11 | BC1F3-11 | 230 | 220 | 240 |
| 12 | BC1F3-12 | 230/250 | 220/230 | 230/240 |
| 13 | BC1F3-13 | 230 | 220 | 240 |
| 14 | BC1F3-14 | 230/250 | 230 | 240 |
| 15 | BC1F3-15 | 230/250 | 220/230 | 230 |
| 16 | BC1F3-16 | 230/250 | 220/230 | 240 |
| 17 | BC1F3-17 | 230/250 | 230 | 230 |
| 18 | BC1F3-18 | 230 | 220/230 | 230/240 |
| 19 | BC1F3-19 | 230 | 220/230 | 230/240 |
| 20 | BC1F3-20 | 230 | 230 | 240 |
| 21 | BC1F3-21 | 230 | 220 | 230 |
| 22 | BC1F3-22 | 250 | 220 | 240 |
| 23 | BC1F3-23 | 230/250 | 220 | 240 |
| 24 | BC1F3-24 | 230/250 | 220/230 | 240 |
| 25 | BC1F3-25 | 230/250 | 230 | 230/240 |
| 26 | BC1F3-26 | 230/250 | 220 | 230/240 |
| 27 | BC1F3-27 | 230 | 220 | 230 |
| 28 | BC1F3-28 | 230 | 220 | 230 |
| 29 | BC1F3-29 | 230 | 230 | 230 |
| 30 | BC1F3-30 | 230 | 220 | 230 |
| 31 | BC1F3-31 | 230 | 230 | 240 |
| 32 | BC1F3-32 | 230/250 | 230 | 230/240 |
| 33 | BC1F3-33 | 230 | 220 | 230 |
| 34 | BC1F3-34 | 230/250 | 230 | 230 |
| 35 | BC1F3-35 | 230 | 230 | 230/240 |
| 36 | BC1F3-36 | 250 | 220 | 230 |
| 37 | BC1F3-37 | 230 | 230 | 240 |
| 38 | BC1F3-38 | 230 | 220/230 | 230 |
| 39 | BC1F3-39 | 230 | 230 | 230 |
| 40 | BC1F3-40 | 230 | 230 | 230/240 |
| 41 | BC1F3-41 | 230/250 | 220 | 240 |
| 42 | BC1F3-42 | 250 | 220 | 230 |
| 43 | BC1F3-43 | 250 | 220 | 240 |
| 44 | BC1F3-44 | 230/250 | 230 | 230/240 |
| 45 | BC1F3-45 | 230 | 230 | 230 |
| 46 | BC1F3-46 | 230 | 230 | 230 |
| 47 | BC1F3-47 | 230/250 | 230 | 230/240 |
| 48 | BC1F3-48 | 230/250 | 220 | 230 |
| 49 | BC1F3-49 | 230 | 220/230 | 230/240 |
| 50 | BC1F3-50 | 230/250 | 230 | 230/240 |
| 51 | BC1F3-51 | 250 | 230 | 230/240 |
| 52 | BC1F3-52 | 230/250 | 220 | 230 |
| 53 | BC1F3-53 | 250 | 230 | 230 |
| 54 | BC1F3-54 | 250 | 220 | 230 |
| 55 | BC1F3-55 | 250 | 220/230 | 230 |
| 56 | BC1F3-56 | 250 | 230 | 230 |
| 57 | BC1F3-57 | 250 | 230 | 230/240 |
| 58 | BC1F3-58 | 230 | 220/230 | 230/240 |
| 59 | BC1F3-59 | 230/250 | 220/230 | 230/240 |
| 60 | BC1F3-60 | 230/250 | 220 | 230 |
| 61 | BC1F3-61 | 230/250 | 220 | 230 |
| 62 | BC1F3-62 | 230 | 220 | 230 |
| 63 | BC1F3-63 | 230 | 220 | 230 |
| 64 | BC1F3-64 | 230 | 220 | 240 |
| 65 | BC1F3-65 | 230 | 220/230 | 230 |
| 66 | BC1F3-66 | 230/250 | 220 | 240 |
| 67 | BC1F3-67 | 250 | 220 | 240 |
| 68 | BC1F3-68 | 230/250 | 220/230 | 230/240 |
| 69 | BC1F3-69 | 230 | 220 | 240 |
| 70 | BC1F3-70 | 250 | 220 | 230/240 |
| 71 | BC1F3-71 | 230/250 | 220/230 | 230/240 |
| 72 | BC1F3-72 | 250 | 220/230 | 230/240 |
| 73 | BC1F3-73 | 230 | 220/230 | 230 |
| 74 | BC1F3-74 | 250 | 220/230 | 230/240 |
| 75 | BC1F3-75 | 230 | 220/230 | 230 |
| 76 | BC1F3-76 | 230/250 | 220/230 | 230/240 |