Introgression of anthracnose resistance Co-4 gene in elite French bean (*Phaseolus vulgaris* L.) background using linked molecular marker SY20

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Abstract

French bean is one of the most important staple foods in many parts of the world. Among several bacterial, fungal and viral diseases, anthracnose caused by *Colletotrichum lindemuthianum* is the most widespread and severe disease of French bean. Therefore, the present study was undertaken with the objective of introgression of important bean anthracnose resistance gene *Co-4* in elite cultivar. Hybridisation between resistant genotype, TO having bean anthracnose resistance gene *Co-4* and elite cultivar Arka Komal was done. Cross Arka Komal × TO was further advanced to generate F₁, BC₁F₁, BC₂F₁ and BC₂F₂ plants. Testing of hybridity of the resultant F₁, BC₁F₁, BC₂F₁ and BC₂F₂ plants/pods using gene linked SCAR marker SY20 resulted in identification of 46 gene-positive BC₂F₂. Further foreground selection revealed lack of segregation for the target gene *Co-4* in 16 progenies. Screening of progenies following detached pod and germinated seed dip methods using race 3 of *C. lindemuthianum* validated *Co-4* imparted resistance in all the 16 BC₂F₃ progenies. Agronomic evaluation of 16 *Co-4* gene-positive BC₂F₄ progenies for 8 qualitative traits and 9 quantitative traits identified AKTO 4, AKTO 5, AKTO 7 and AKTO 43 progenies as having better elite background. These can either be used as donors of resistance gene for further introgression and gene pyramiding or can further be advanced following backcrossing to develop essentially derived variety of elite parent, Arka Komal.

Introduction

French bean belongs to order Fabales and family Fabaceae, sub family *Papilionideae*, tribe *Phaseoleae*, sub-tribe *Phaseolionae* and genus *Phaseolus*. French bean is an autogamous diploid (2n = 2x = 22) species with a comparatively small genome ≈ 587 mbp (Bennett and Leitch 1995; Broughton et al. 2003) and genetic consensus map is structured into 11 chromosomes (Nodari et al. 1993; Freyre et al. 1998; Pedrosa-Harand et al. 2008). Beans comprises of two major eco-geographical gene pools, Middle America and the Andes (Kwak and Gepts 2009). French bean is a rich and low-cost source of protein, fiber and other important minerals, i.e. iron, zinc, copper, phosphorus, potassium, magnesium, calcium and many vitamins (Limongelli et al. 1996; Kaur et al. 2009; Khetan et al. 2015). Therefore, the crop is considered an economically, nutritionally and socially important (Broughton et al. 2003; Pujola et al. 2007; Mishra et al. 2010; Singh et al. 2012). Green beans are cultivated throughout the world over an area of about 1.56 million hectares with a production and productivity of 23.60 million tonnes and 15.15 tonnes/ha, respectively (FAO, 2016). Latin America is the largest producer with Brazil and Mexico being by far the major producers. Africa is the second most important region, producing about 2.5 million metric tons, with Uganda, Kenya, Rwanda, Burundi, Tanzania and Congo playing major roles (CGIAR French bean, 2012). In India, green beans are cultivated over an area of 241 thousand hectares with a production and productivity of about 675 thousand tonnes and 2.80 tonnes/ha, respectively (FAO, 2017).

Several bacterial, fungal and viral diseases attack French bean and among these anthracnose caused by fungus *Colletotrichum lindemuthianum* is one of the most widespread and severe. This disease can cause yield losses of up to 100% when infected seed is sown in areas with high relative humidity and mild temperatures range from 18 to 24 °C (Peloso 1992). Majority of the recommended and locally adapted bean cultivars in Himachal Pradesh are susceptible to different races of pathogen (Sharma et al. 1999). The most prevalent races in the region are race 3, 115, 513, 515, 529, 537, 615, 631, 775 and 935 (Pathania et al. 2006). Studies on race spectrum of bean anthracnose have revealed presence of 27 races in all from North Western Himalayan region, out of which 19 are reported to be present in Himachal Pradesh (Sharma et al. 2019) Both cultural management and use of fungicides can be followed to control the disease. However, chemical control is found to be expensive and generation of pathogen free seeds is often difficult in developing countries. So, the use of resistant genotypes emerges to be the most biologically safe and cost-effective management strategy (Pastor-Corrales and Tu 1989). A large number of studies on genetics of resistance to anthracnose have revealed it to be governed by 26 anthracnose resistance loci identified and denoted by the symbol *Co* including *Co-1* to *Co-7*, *co-8*, *Co-9* to *Co-18*, *Co-u* to *Co-z* (Kelly and Vallejo 2004; Goncalves-Vidigal et al. 2012), *CoPv09* (Campa et al. 2014) and *Co-Pa* (de Lima Castro et al. 2017). Many improved pencil type varieties developed by public and private sectors are available for cultivation by the farmers of the state however most of these are susceptible to many prevalent races of bean anthracnose. Arka Komal is such preferred bush type French bean variety grown by the farmers of Himachal Pradesh and its adjoining hilly and plain areas which are highly susceptible to this disease. Thus there is need to develop French bean variety having resistance to bean anthracnose to counter threats of this disease on bean production. Therefore, the present study is proposed with the objectives of introgression of important bean anthracnose resistance gene *Co-4* in elite cultivar and to assess the derivatives for validation of imparted resistance. The study will result in identification of anthracnose resistant derivatives which can further be advanced to isolate derived variety (ies) and used as prebreeding material in bean anthracnose breeding programme.

Materials And Methods

Plant material

The French bean genotype TO (*Co-4*) was used as resistance donor against bean anthracnose, whereas French bean Arka Komal was used as recipient parent.

Hybridisation and backcrossing to generate F₁, BC₁F₁ and BC₂F₁ seeds

The hybridization was attempted between bean anthracnose resistance gene donor genotype TO and elite cultivar Arka Komal gene donor as male parent. The F₁ progenies (Arka Komal × TO) were then backcrossed with respective elite parent using F₁ as male parent. The process of hybridization involved emasculation of the flowers and artificial pollination of emasculated flowers next morning.

The data on total number of flowers emasculated and pollinated and total number of pods set were collected cross-wise. Crossability was expressed as the per cent pod set in hand pollinated flowers and was calculated as follows:

Crossability (%) = (Total number of pods set / Total number of flowers pollinated) × 100

Marker assisted foreground selection

The DNA for marker analysis was extracted by CTAB method (Murray and Thompson, 1980). The SCAR marker *SY20* reported to be linked to the targeted bean anthracnose resistance gene *Co-4* was used to identify the plants carrying the resistance gene in F₁, BC₁F₁, BC₂F₁, BC₂F₂ and BC₂F₃ progenies.

Isolation, purification and quantification of the plant genomic DNA

Genomic DNA of plants of parents and various generations were isolated from young leaves using CTAB method (Murray and Thompson 1980). The extracted DNA samples were loaded on 1% agarose gel (1gm/100ml 1X TAE Buffer) and run at 90 V for 30 mins to ascertain the quality and quantity of DNA.

PCR amplification

DNA samples were used for PCR amplification by using *SY20* SCAR primer (F: AGCCGTGGAAGGTTGTCAT, T_m - 56.9°C; R: CCGTGGAAACAACACACAAT, T_m - 53.9°C). For amplification of genomic DNA, a reaction mixture was prepared using PCR water, DNA (15-25 ng/μl), 10X buffer (10 mM), dNTP mix (0.5 mM each of dATP, dGTP, dCTP, dTTP), forward primer (5 pM), reverse primer (5 pM) and *Taq* polymerase (3 U/μl). The amplification was carried out in Veriti 96 wells Thermal Cycler (Applied Biosystem, CA, USA) following PCR amplification profile for *Co-4*. The PCR cycle for *SY20* scar marker consisted of 2 min at 94°C; followed by 30 cycles of 30 s at 94°C, 1 min at 59.2°C and 1.5 min at 72°C; a 5 min extension at 72°C; and a hold at 4°C.

Analysis of PCR products

For analysis of PCR products, 2 % agarose gel was prepared in 1X Tris-Acetate-EDTA and ethidium bromide (0.5μg/ml) was added to gel solution before setting the gel on casting plate. The PCR products (10 μl) along with 2μl loading dye (0.25 % bromophenol blue and 40 % sucrose) were loaded in the gel inside the electrophoresis tank along with a 100 bp DNA ladder and resolved on 2 % agarose gel. Electrophoresis was carried out at 100-120 V for 90 mins and finally the gel was visualised and photographed using the Gel–Documentation Unit (Labnet, ENDURO™ GDS, Aplegen). Presence/absence of appropriate size product of the marker *SY20* (830 bp) linked to the anthracnose resistant gene, *Co-4* was recorded to infer the presence of *Co-4* gene in the parents and progenies.

Validation of anthracnose resistance

Cultures of *C. lindemuthianum* race-3 showing virulence on susceptible parent and avirulence on *Co-4* gene harbouring resistant parent To, were used for screening and validation of *Co-4* imparted anthracnose resistance in homozygous selected lines harbouring resistance genes. Validation of resistance was done following detached pod and germinated seed dip method.

Screening by detached pod method was repeated twice to confirm the disease reactions. Infection type on inoculated pods was evaluated for disease severity by following the rating scale of 0-9 (Mayee and Dattar 1986) as given below, where

0= No disease on pods

1= Minute flecks on pods

3= Brown sunken spots, < 1 per cent pod area

5= Brown spots, 1-10 per cent pod area

7= Circular brown sunken spots, 11-25 per cent pod area

9= Circular to irregular, > 26 per cent pod area

Pods scoring reaction types of 0, 1 and 3 were graded as resistant while those scoring 5, 7 and 9 were graded as susceptible.

In seed dip method the reactions of inoculated seedlings were scored after six and twelve days of inoculation following 0-5 scale (Drijfhout and Davis 1989), where

0 = No disease

1 = Pin point lesions

2 = Small lesions, not sunken

3 = large sunken lesions

4 = large, deep lesions up to stem centre

5 = seedling killed by the pathogen

Plants scoring reaction types of 0, 1 and 2 were graded as resistant while those scoring 3, 4 and 5 were graded as susceptible.

Agronomic evaluation of selected *Co-4* gene-positive anthracnose resistant homozygous derivatives

Experimental site, material and layout design

The agronomic evaluation of 16 selected *Co-4* gene-positive homozygous BC₂F₄ derivatives exhibiting anthracnose resistance was conducted under protected cultivation facilities (cage-house). The site is situated at an elevation of 1,290.8 m above mean sea level with 32° 6' N latitude and 76° 3' E longitude. Seeds of 16 selected *Co-4* gene-positive homozygous derivatives (BC₂F₄ seeds) along with both parents *i.e.* Arka Komal and TO were planted for evaluating the agronomic and horticultural traits. Parents along with gene-positive homozygous derivative lines were planted in a replicated trial following Completely Randomized Block Design (CRBD) in single rows of 1.25 m each replicated thrice. Recommended cultural practices were followed to raise the crop.

Data recording

Data were recorded on randomly selected 5 plants in each line/progeny and replication on the following 8 qualitative and 9 quantitative important agro-horticultural traits each as per the details given below:

Qualitative traits

Growth habit of plant

The growth habit of the plants was characterized as trailing, intermediate trailing, bush type and segregating recorded on each progeny/line.

Pod shape

The shape of pods of all plants of each line was observed visually and categorized on majority basis as curved, moderately curved, slightly curved and straight.

Pod colour at picking stage

The pod colour at picking stage was observed visually and categorized on majority basis as green.

Stem colour

The stem colour was observed visually and categorized on majority basis as green.

Seed coat pattern

The seed coat pattern of the seeds was characterized as mottled and absent on each homozygous progeny/line.

Seed coat colour

The seed coat colour of the seeds was observed visually and categorized as different ranges from greyish white to brown, greyish white to dark brown, greyish white to light brown, greyish white to beige, greyish white to light beige, greyish white to golden brown, greyish white to pinkish brown and greyish white.

Presence of colour on around of hilum

The presence and absence of colour on around of hilum was observed visually on each homozygous derivative line along with parents.

Brilliance of seeds

The brilliance of seeds was characterized as medium and shiny on each homozygous progeny/line along with parents.

Quantitative traits

Days to flowering initiation

Days from date of sowing to the day when first flower appeared on the plants of line were recorded for each line in each replication.

Days to 50 per cent flowering

Days from date of sowing to the day when almost all plants had 50 per cent flowering were recorded for each line in each replication.

Plant height at 50 per cent flowering (cm)

Height of 5 randomly selected plants of each line was measured at 50 per cent flowering stage from ground to tip of plant and the mean recorded as plant height in cm for each row in each replication.

No. of branches/plant

Branches present on each 5 individual randomly selected plants were counted and mean recorded for each line in each replication.

No. of pod bearing nodes/plant

Number of pod bearing nodes was counted on all 5 individual selected plants and average recorded for each line in all replications.

Pods/node

Pods present on all pod bearing nodes of the selected 5 plants in each line were counted and average recorded for each line in all replications.

Pods/plant

Total green tender pods picked from selected 5 plants under different pickings were counted and mean recorded for each line in all replications.

Pod length (cm)

Length of the ten randomly chosen green pods picked from 5 selected plants was measured and average recorded in cm for each line in all replications.

Green pod yield/plant (g)

The green pod yield obtained from the 5 randomly selected plants under different pickings was summed and average recorded in grams (g) for each line in all replications.

Statistical analysis

The data collected on 9 quantitative traits were subjected to analysis of variance (ANOVA) following standard statistical procedure following Panse and Sukhatme (1984) as per the model and ANOVA given below:

$$Y_{ij} = \mu + g_i + r_j + e_{ij}$$

where,

$$Y_{ij} = \text{Phenotypic observation of the } i\text{th entry in the } j\text{th replicate,}$$

$$\mu = \text{General mean,}$$

$$g_i = \text{Effect of } i\text{th entry,}$$

$$r_j = \text{Effect of } j\text{th replication, and}$$

$$e_{ij} = \text{Error component.}$$

Analysis of variance

Source	Df	Mean squares	Expected mean squares
Replication	(r-1)	M_r	$\sigma_e^2 + g \sigma_r^2$
Entries	(g-1)	M_g	$\sigma_e^2 + r \sigma_g^2$
Error	(r-1) (g-1)	M_e	σ_e^2

where,

$$r = \text{Number of replications,}$$

$$g = \text{Number of entries/genotypes,}$$

$$\sigma_e^2 = \text{Error variance,}$$

$$\sigma_r^2 = \text{Variance due to replications and}$$

$$\sigma_g^2 = \text{Variance due to entries.}$$

The entries/genotypes mean sum of squares were tested against error mean squares by “F” test at (g-1) and (r-1) × (g-1) degree of freedom at $P \leq 0.05$ for statistical significance of differences among genotypes/entries. Based on the above ANOVA, the following

statistical parameters were calculated for inference of the data and its interpretations:

$$\text{Standard Error of mean (SE}_m\text{)} = \sqrt{M_e/r}$$

$$\text{Standard Error of difference (SE}_d\text{)} = \sqrt{2M_e/r}$$

$$\text{Critical Difference (CD) at } P \leq 0.05 = SE_d \times \text{tabulated 't' value at error df}$$

$$\text{Coefficient of Variation (CV \%)} = (\sigma/\mu) \times 100$$

where,

$$\mu = \text{General mean,}$$

$$M_e = \text{Mean error sum of squares,}$$

$$r = \text{Number of replications, and}$$

$$\sigma = \text{Standard deviation.}$$

Results

Hybridisation and backcrossing to generate F₁, BC₁F₁ and BC₂F₁ seeds

Bean anthracnose resistant genotype, TO known to harbour bean anthracnose resistance gene, *Co-4* was crossed with elite cultivar, Arka Komal known to be susceptible to the disease. Resistant genotype was used as male parent to generate F₁ seeds. For the cross Arka Komal × TO, 104 flowers were emasculated and pollinated. Out of these only eleven pollinations were successful leading to development of 11 (8 were validated by marker) revealing thereby a crossability rate of 7.69% under Palampur cage house conditions.

Validation of hybridity of developed F₁ seeds

At the young stage, hybridity of 11 pod progenies of cross Arka Komal × TO was validated using *Co-4* linked SCAR marker, *SY20*. The results obtained are presented in Fig. 1. Out of 11 representative plants of each developed pod only eight exhibited presence of desirable amplicon (830 bp) of molecular marker, *SY20* linked to the gene *Co-4* indicating the hybrid nature, whereas amplification of marker failed in case of three plants *i.e.* plant number 3, 9 and 10. The likelihood of such pods developing as a result of selfing can be due to misjudgement in identifying appropriate stage flowers and/ a result of leftover anthers in the emasculated flowers. Selected gene-positive 8 F₁ plants of cross Arka Komal × TO were used for backcrossing with elite parent. Overall 68 flowers of Arka Komal were emasculated and pollinated with pollens of the 8 hybrid plants to develop BC₁F₁ progenies during the season resulting in development of 3 putative BC₁F₁ pods. The foreground selection using *SY20* for Cross {Arka Komal × F₁ (Arka Komal × TO)} indicated that out of 3 putative BC₁F₁ plants, representative plants of all three pods exhibited desired amplicon of 830 bp and were thus inferred to be gene-positive and true BC₁F₁ (Fig. 2). Selected 3 gene-positive BC₁F₁ plants were used for further backcrossing with the elite parent, Arka Komal. To develop BC₂F₁ progenies 96 flowers of Arka Komal were emasculated and pollinated with pollen of the 3 selected gene-positive BC₁F₁ plants during the season - III resulting in development of 6 BC₂F₁ pods. The foreground selection using *SY20* for cross [Arka Komal × BC₁F₁{Arka Komal × F₁ (Arka Komal × TO)}] indicated that out of 6 putative BC₂F₁ plants, only two plants exhibited desired amplicon of 830 bp and were thus inferred to be gene-positive and true BC₂F₁ (Fig. 3). The remaining 4 pods were inferred to be self and hence not advanced further.

Selfing of BC₂F₁ plants to develop BC₂F₂ seeds and selection of gene-positive BC₂F₂ plants

After molecular validation of the BC₂F₁ plants, gene-positive plants were further advanced through selfing to develop BC₂F₂ seeds. These BC₂F₂ seeds were grown in the cage house in the subsequent season. A total of 75 BC₂F₂ seeds were grown in the cage house, out of which 68 seeds germinated. These 68 plants were then validated using gene-linked molecular markers which exhibited desired amplicons of 830 linked to *Co-4*. The detailed results are depicted in Fig. 4. The results of the foreground selection in these

plants revealed that for out of a total population of 68 BC₂F₂ plants, 46 plants (Plant Nos. 1, 2, 3, 4, 5, 6, 8, 10, 11, 12, 16, 17, 18, 19, 21, 23, 24, 25, 26, 28, 29, 32, 33, 34, 35, 36, 37, 38, 39, 41, 43, 45, 48, 51, 52, 53, 54, 56, 57, 58, 59, 62, 63, 66, 67 and 68) were inferred to be *Co-4* gene-positive. Overall the observed segregation ratio of gene-positive and gene-negative plants was 67.65% and 32.35% as against the expected perfect fit of 75% and 25%, respectively.

Selection of homozygous BC₂F₃ derivatives for target gene *Co-4*

The seeds of gene-positive plants identified above were harvested as single plants and advanced as plant to row progenies to develop BC₂F₃ plants. Ten seeds each of 46 BC₂F₃ plant progenies were sown as rows (Progeny 1 to 46) in the cage house. The information on total number of seeds harvested from each BC₂F₂ gene-positive plant, number of seeds sown, germinated and segregation status for the target gene in each plant to row progeny of cross is presented in Table 1.

A total of 1259 BC₂F₃ seeds were harvested from 46 selected gene-positive BC₂F₂ plants, out of which 9-10 seeds of each progeny were sown as single rows. A total of 457 seed of 46 BC₂F₂ plants were sown in 46 progeny rows out of which 427 germinated exhibiting a high seed germination of 93.4 % in BC₂F₃ seeds. Foreground selection with linked marker, *SY20* in all the plants of the progeny row revealed lack of segregation in 16 progenies out of the total of 46 screened including 4, 5, 7, 8, 10, 19, 24, 28, 29, 33, 35, 40, 42, 43, 45 and 46. These were inferred to be homozygous for the target gene, *Co-4*. Notably seed germination in these 16 progenies was observed cent per cent. In the remaining 30 progenies, segregation for the linked marker was observed. Progeny 1, 2, 3, 6, 9, 11, 12, 13, 16, 17, 18, 20 and 21 exhibited cent per cent germination and foreground selection indicated that out of 10 BC₂F₃ plants in each row, 8, 6, 8, 9, 6, 7, 7, 9, 7, 7, 8, 7 and 6 plants, respectively exhibited presence of desired amplicon and were thus inferred to be heterozygous. In progeny 14, 15, 26, 27, 36 and 38, seed germination was observed 90 per cent and foreground selection showed that out of 9 BC₂F₃ plants in each row, 8, 8, 8, 7, 8 and 7 plants, respectively exhibited desired amplicon of 830 bp. In progeny 22, 23, 25, 31, 32, 37 and 41 comparatively lower seed germination of 80 % was observed and out of 8 BC₂F₃ plants in each row, only 7, 7, 7, 6 and 6 plants, respectively were gene-positive when foreground selection was done by using *SY20* SCAR marker. In progeny 30, 34, 39 and 44 showed only 4, 5, 6 and 6 BC₂F₃ plants, respectively in each row were gene-positive. Agarose gel showing presence/absence of amplification products (830 bp bands) of SCAR markers, *SY20* in the parents and 427 BC₂F₃ plants in each row are depicted in Fig. 5, 6 and 7.

Validation of *Co-4* imparted anthracnose resistance by using cultures of race 3 of *C. lindemuthianum*

Validation of resistance in 16 selected homozygous lines for *Co-4* gene was done by following detached pod and germinated seed dip method (repeated twice). Culture of *C. lindemuthianum* race-3 showing virulence on susceptible parent and known to be avirulent on *Co-4* gene was used for screening and validation of anthracnose resistance of 16 selected homozygous lines carrying *Co-4* gene as inferred by amplification of gene linked SCAR marker. Results of screening using both methods are presented in Table 2 and disease symptoms/reactions shown in Figure 7 (detached pod method) and Figure 8 (germinated seed dip method).

Screening for bean anthracnose using detached pod method revealed a disease score of '5' in the recipient parent, Arka Komal, whereas in resistant donor TO a disease score of '0' was recorded. All the 16 BC₂F₃ progenies inferred to be homozygous for the target gene *Co-4*, recorded a disease score of '0' revealing there by resistant reaction of donor and 16 backcross progenies. Germinated seed dip method confirmed these results wherein also disease score of '0' was recorded in donor parent TO and 16 backcross progenies. These results clearly validated the presence of anthracnose resistance gene *Co-4* selected through linked marker *SY20* and *Co-4* gene imparted resistance to race 3 of *C. lindemuthianum* in all 16 selected progenies.

Agronomic evaluation of the selected 16 BC₂F₄ progenies carrying *Co-4* gene for 8 qualitative traits

Agronomic evaluation for eight qualitative traits, scored visually revealed that all 16 *Co-4* gene-positive homozygous lines were fixed for bushy growth habit as none of plant progenies exhibited segregation for the trait, whereas susceptible parent, Arka Komal has bush type growth habit and donor, parent TO (*Co-4*) has intermediate trailing habit. Pod shape of recipient parent, Arka Komal was straight/ slightly curved/ moderately curved and that of the donors, TO was straight/ slightly curved. Test cultures exhibited wide variation for pod shape ranging from straight to curved pods. Pods of *Co-4* gene-positive homozygous sixteen progenies were graded straight in 4 progenies, namely AKTO 19, AKTO 28, AKTO 33 and AKTO 36 whereas pods of other 10 progenies, AKTO 4, AKTO 8, AKTO 10, AKTO 24, AKTO 29, AKTO 40, AKTO 42, AKTO 43, AKTO 45 and AKTO 46 were graded slightly curved. Pods of 2

progenies, AKTO 5 and AKTO 7 were graded as moderately curved and some pods in AKTO 7 had curved pods along with moderately curved.

Pod colour at physiological maturity stage was noticed visually and pod colour of parent, Arka Komal and TO was observed green. All 16 *Co-4* gene-positive progenies exhibited green pod colour at physiological maturity stage. Stem colour of parents and backcross progenies revealed that Arka Komal and donor, parent TO (*Co-4*) has green stem colour. All 16 *Co-4* gene-positive backcross progenies also exhibited green stem colour. There is absence of any seed coat pattern in the seeds of recipient parent, Arka Komal whereas seed coat pattern of the donor parent, TO was observed to be mottled. All 16 *Co-4* gene-positive back cross progenies were observed to have mottled seed coat pattern ranging from sparse to dense brown in colour. Seed coat colour of recipient parent, Arka Komal was observed grayish white to brown and donor parents, TO were noticed grayish white to beige. Seed coat colour of *Co-4* gene-positive back cross progenies exhibited a large variation ranging from grayish white to beige, grayish white to light beige, grayish white to dark brown, grayish white to golden brown, grayish white to pinkish brown and golden brown (Table 3). Presence of colour on around of hilum was observed in recipient parent, Arka Komal and donor parent, TO. The presence of brown colour around the hilum was noticed in all sixteen *Co-4* gene positive homozygous rows. Seeds brilliance of recipient parent, Arka Komal was observed shiny whereas donor parent, TO showed medium brilliance of seeds. Out of sixteen *Co-4* gene-positive homozygous progenies, 5 (AKTO 29, AKTO 36, AKTO 40, AKTO 42 and AKTO 43) were found with shiny brilliance of seeds and 11 progenies (AKTO 4, AKTO 5, AKTO 7, AKTO 8, AKTO 10, AKTO 19, AKTO 24, AKTO 28, AKTO 33, AKTO 45 and AKTO 46) were observed to have medium seed brilliance.

Agronomic evaluation of the selected 16 BC₂F₄ progenies carrying *Co-4* gene for 9 quantitative traits

Analysis of variance

The analysis of variance of data recorded on 9 quantitative agro-horticultural traits, viz. days to flowering initiation, days to 50 per cent flowering, plant height at 50 per cent flowering (cm), number of branches/plant, number of pod bearing nodes/plant, pods/node, pods/plant, pod length (cm) and green pod yield/plant (g) revealed that mean sum of squares due to genotypes/treatments were significant for all the nine traits studied in the backcross progenies revealing thereby sufficient variation among test cultures for all the traits (Table 4 and 5).

Performance of the parents and 16 *Co-4* gene-positive BC₂F₄ progenies

Agronomic evaluation for 9 agro-horticultural traits revealed that the susceptible parent, Arka Komal is early flowering (35 days), whereas donor parents TO took 42 days for flowering initiation. Most of the *Co-4* gene-positive homozygous progenies took intermediate time for flowering initiation that ranged from 36-42 days and none of these were found to be earlier flowering than the early parent, Arka Komal. However, 7 lines, AKTO 8, AKTO 10, AKTO 19, AKTO 24, AKTO 28, AKTO 42 and 43 were at par for duration of flowering initiation with early parent, Arka Komal. Remaining 9 *Co-4* gene-positive derivatives exhibited significantly late flower initiation as compared to elite parent, Arka Komal (Table 5).

Fifty per cent flowering occurred in 37 days in recipient parent, Arka Komal and in 44 days in the donor parents, TO. Duration of 50 per cent flowering ranged from 38-44 days in the 16 *Co-4* gene-positive backcross progenies. Most of the these took longer than early parent, Arka Komal for 50 per cent flowering except progenies AKTO 5, AKTO 10, AKTO 24, AKTO 28 and AKTO 42 that were statistically at par with the early flowering parent, Arka Komal.

The mean plant height of recipient parental line, Arka Komal at 50 per cent flowering was 45.83 cm whereas that of the donor parent, TO was 80.17 cm as this line have trailing type of growth habit. Most of the *Co-4* gene-positive backcross progenies (10) exhibited shorter plant height as compared to the elite parent, Arka Komal ranging from 30.83 to 42.33 cm. Progenies, AKTO 4, AKTO 8, AKTO 10, AKTO 19 and AKTO 24 had plant height at par with dwarf parent, Arka Komal. Progenies AKTO 7 and AKTO 43 were taller as compared to dwarf parent Arka Komal.

The mean branches/plant in the recipient parent, Arka Komal was 4.50 whereas the donor parent TO had 1.83 branches/plant. Mean number of branches/plant in *Co-4* gene-positive backcross progenies varied from 2.50 to 4 in the 16 progenies. Seven progenies, AKTO 7, AKTO 8, AKTO 10, AKTO 29, AKTO 36, AKTO 40 and AKTO 45 had branches/plant at par with the elite parent, whereas remaining 9 progenies had lesser branches/plant than the better parent, Arka Komal.

Mean pod bearing nodes/plant in the parental lines, Arka Komal and TO was 4.83 and 5.33, respectively. Pod bearing nodes/plant in the *Co-4* gene-positive backcross progenies ranged from 4.17 to 8.0 revealing thereby a wide range of variation for this trait. Progenies, AKTO 4 and AKTO 43 had significantly better pod bearing nodes/plant as compared to the better parent. Rest of the 14 progenies exhibited pod bearing nodes/plant at par with the elite parent, Arka Komal.

There was a narrow range of variation for mean number of pods/node. Arka Komal exhibited higher number of pods/node (2.50) as compared to donor parents TO (1.50). Thirteen *Co-4* gene-positive back cross progenies out of 16 exhibited pods/node at par with the recipient parent, Arka Komal except progenies AKTO 7, AKTO 8 and AKTO 43 that had better pods/node than the elite parent, Arka Komal.

Average pod length in the recipient parental line Arka Komal was highest (15.83 cm) whereas in TO it was 9.53 cm. The pod length of the *Co-4* gene-positive progenies ranged from 11.37 to 14.20 cm and pods of all the progenies were shorter in length than the pods of better parent, Arka Komal. All the progenies exhibited pod length better than the *Co-4* gene donor parent, TO.

Average pods/plant in recipient parent line, Arka Komal was 10.17 whereas in donor parent, TO it was 12.50. In donor parent TO the pods/plants were higher which might be due to its indeterminate and trailing growth habit. In *Co-4* gene-positive back cross progenies the pods/plant varied from 6.17 to 14.67. Nine progenies, AKTO 4, AKTO 7, AKTO 8, AKTO 19, AKTO 24, AKTO 29, AKTO 33, AKTO 40 and AKTO 45 were at par with the elite parent Arka Komal. Two progenies AKTO 5 and AKTO 43 had significantly higher pods/ plant than the elite parent, Arka Komal whereas remaining 5 progenies were inferior to both the parents. *Co-4* gene donor parent, TO had better pods/plant and none of back cross progenies surpassed it for pods/plant.

Green pod yield/plant of the recipient parental line, Arka Komal was 67.67 g and that of donor parent, TO from three pickings was 35 g, whereas *Co-4* gene-positive back cross progenies had green pod yield/plant ranging from 28.33-65.0 g. Out of 16 progenies only 2 (AKTO 7 and AKTO 43) yielded at par with better parent, Arka Komal, whereas in the remaining 14 progenies, green pod yield/plant was lower than the elite parent.

Based on performance of 16 backcross progenies for all the traits, four best progenies performing at par with better parent and progenies performing better than elite parent were identified and are presented in Tables 6. Overall agronomic performance indicated *Co-4* gene-positive progenies AKTO 10, AKTO 19, AKTO 24 and AKTO 42 are the 4 best progenies for days to flower initiation that are at par with elite parent Arka Komal (Table 6). Similarly progenies AKTO 5, AKTO 10, AKTO 24 and AKTO 42 were the best four early flowering at par with the elite parent and hence can be used as donor of earliness along with resistance gene, *Co-4*. Progenies, AKTO 4, AKTO8, AKTO 10 and AKTO 24 were at par with elite parent for plant height. Progenies, AKTO 7, AKTO 10, AKTO 36 and AKTO 40 were at par with elite parent for branches/plant. Similarly AKTO 5, AKTO 29, AKTO 40 and AKTO 46 were at par with elite parent for pod bearing nodes/plant whereas AKTO 4 and AKTO 43 were better than elite parent, Arka Komal. For pods/node, progenies AKTO 7, AKTO 8 and AKTO 43 were better than elite parent whereas progenies AKTO 5, AKTO 19, AKTO 24 and AKTO 43 were four best at par with elite parent. Progenies, AKTO 5 and AKTO 43 had better pods/plant than elite parent whereas progenies AKTO 7, AKTO 8, AKTO 33 and AKTO 40 were four best at par with elite parent. Only 2 progenies AKTO 7 and AKTO 43 were comparable in pod yield with elite parent. Overall 2 progenies namely AKTO 7 and AKTO 43 exhibited better pod bearing nodes/plant, pods/node and pods/plant and thus exhibited better green pod yield/plant comparable with elite parent Arka Komal.

Discussion

The crossability relationships between and within *Phaseolus* species have been extensively studied and reviewed (Smartt 1990; Debouck and Smartt 1995). Generally, there are strong barriers for interspecific hybridisation in *Phaseolus*, but between each cultigens and its wild ancestral type, almost full genetic compatibility exists (Mumba and Galwey 1998). Mumba and Galwey (1998) in their exhaustive study on cross compatibility between gene pools and evolutionary groups in *Phaseolus vulgaris* L. has clearly demonstrated that there is no general consistent tendency for pollinations between genotypes within a gene pool to be more successful than those between gene pools. In evolutionary groups (wild, landrace and bred), the crosses among landrace and bred genotypes, those within evolutionary classes had higher success rates than those between classes (47.2% vs. 38.9%).

In the present study, the extent of crossability recorded (7.69%) can easily be considered as comparatively low as the parent of the present study belong to the same evolutionary group *i.e.* bred genotypes should have shown higher compatibility as reported by the

aforementioned studies. However, the efficiency of crossing depends on the fact that the stigma and pollen should be in very close contact as long as possible to ensure fertilization, as occurs with in the keel in natural selfing. Low cross compatibility in the present case can probably be ascribed to comparatively unfavorable factors for hybridization mentioned above under cage house as well as to the limited skill of person handling hybridisation. Similarly experience in manual hybridisation is likely to enhance the success rate of hybridisation programmes.

Overall in cross (AK × TO), the observed segregation ratio of gene-positive and gene-negative plants was 67.65% and 32.35% as against the expected perfect fit of 75% and 25%, respectively. Such minor deviation can be a result of small sample size of population, differential seed development as well as a result of selection of seeds as only well filled seeds were advanced in the present case.

Marker assisted selection (MAS) and marker assisted backcross breeding (MABC) have received much attention as a viable method for crop improvement. In the case of disease resistance genes, molecular markers linked to genes of interest can be used to accumulate R genes in a single cultivar without laborious disease screening (Miklas et al. 2006). There is need to develop common bean varieties having resistance to bean anthracnose to counter threats of this disease on bean production. Chemical control is found to be expensive and generation of pathogen free seeds is often difficult in developing countries. So, the use of resistant genotypes appears to be the most biologically safe and cost-effective management strategy (Pastor-Corrales and Tu 1989).

In common beans, many earlier studies have also reported successful use of linked molecular markers for the selection of different bean anthracnose resistance genes in early as well as advanced segregating material. RAPD markers, OPY20_{830C} (0.0 cM) and OPC08_{900C} (9.7 cM) linked to the *Co-4* gene have been identified by Arruda et al. (2000) in the coupling phase. It has been reported by them that the selection efficiency for resistant and susceptible plants was 100 per cent with RAPD marker OPY20_{830C} due to its close linkage to locus *Co-4* (0.0 ± 1.1 cM) and hence this marker can be used for screening gene harbouring plants in advanced generation (F₆ or later) lines that are nearly homozygous. The SCAR marker, SY20 used in the present study for foreground selection of *Co-4* gene has been developed from this RAPD marker (Queiroz et al. (2004) and therefore is also expected to be equally efficient in indirect selection for the gene, *Co-4*. Beraldo et al. (2009) has validated the specificity of the SY20 marker for the *Co-4* locus while screening 42 parents and 76 elite lines developed by the common bean breeding programme of the *Instituto Agronomico de Campinas* (IAC, Campinas, SP, Brazil) with SY20. In the past successful marker assisted selection has been done by Alzate-Marin et al. (2004) for introgression of *Co-4²* gene with RAPD markers OPH18_{1200C}. Similarly Gu et al. (2011) used five SCAR markers combinations (SCA₁₀₀₀, SH18₁₁₀₀, SAB3₄₀₀, SB12₃₅₀ and SCF10₁₀₇₂) successfully to screen 143 snap bean germplasm for bean anthracnose resistance. Ferreira et al. (2012) reported pyramiding of bean anthracnose resistance genes and resistance to potyvirus in the market class fabada beans using a set of seven molecular markers. Pyramids included genes *Co-2* and *Co-3/9*. Boersma et al. (2013) observed marker assisted dissection of anthracnose resistance in dry bean cultivar, Morden003 previously reported to carry genes, *Co-1*, *Co-2* and *Co-4* on chromosomes Pv01, Pv11 and Pv06, respectively. Sousa et al. (2014) reported that cultivars or elite lines harboring the alleles *Co-4²*, *Co-5* and *Co-6*, individually or in combination (pyramiding), are those with the highest resistance spectrum to anthracnose in Brazil. Moses (2015) used SCAR markers for pyramiding of three bean anthracnose genes (*Co-4²*, *Co-5* and *Co-9*) and one *Pythium* resistance gene into susceptible common bean varieties, K132, NABE4, NABE13 and NABE14 by using a cascading pedigree gene pyramiding scheme. More recently, Manjula et al. (2016) used 15 SCAR markers to identify anthracnose resistance genes in 40 French bean accessions and reported successful amplification of six SCAR markers namely SAS13, SBB14, SC08, SF10, SW12 and SZ04 to detect presence of *Co-4²*, *Co-4*, *Co-10*, *Co-3/Co-9* and *Co-6* genes. Kamiri et al. (2021) tested four SCAR markers in the present work (*SH18* and *SBB-14* for *Co-4²*, *SAB-03* for *Co-5*, and *SY-20* for *Co-4*), have shown to be useful for marker assisted selection of the target resistant genes. They were found specific for the linked *loci*. *SH18* was shown to be specifically linked to *Co-4²* and some other did not discriminate alleles from the same *locus*.

One of the major constraints during breeding for disease resistance is that donor lines are carrier of undesirable attributes that subsequently lead to development of poor performing progenies from crosses of elite parents with gene donors (Hospital 2005). Various approaches are followed to reduce this junk and linkage drag including marker-assisted backcross breeding (MABC) with foreground and accompanying background selection to restore the elite background (Fukuoka et al. 2009). Under such situations, one of the alternative approaches could be pre breeding efforts to bring such important disease resistance genes into comparatively better and/or elite backgrounds, and to use products of such breeding efforts as donors of the genes rather than the original inferior donors.

Studies on race spectrum of bean anthracnose have revealed presence of 27 races in all from North Western Himalayan region, out of which 19 are reported to be present in Himachal Pradesh (Sharma et al. 2019). Apart from race 3, *Co-4* gene is reported to impart resistance against most of the other Indian races including 115, 513, 515, 529, 537, 615 and 631 (Pathania et al. 2006). Out of these 8 races, four new races (115, 537, 615 and 631) have been reported for the first time from Himachal Pradesh, India which had not been reported earlier from the other regions of the world (Sharma et al. 2007). Therefore, deployment of the gene *Co-4* is more relevant in this region as the lines developed under this study harbouring *Co-4* have potential to impart resistance against 8 prevalent races, four of which are new and specific to the region. Therefore, deployment of the gene *Co-4* is most relevant to achieve durable and efficient resistance against bean anthracnose.

In literature, various methods of artificial disease screening for anthracnose resistance are mentioned including seedling inoculation, germinated seed dip inoculation, detached pod and detached leaf methods (Tu 1986). Of these detached leaf and pod method have been reported to be the best by Bigirimana and Hofte (2001). Different methods have associated advantages and disadvantages. In detached leaf/pod method, spores are uniformly distributed over the leaf/pod area which provides accuracy and promptness for screening because disease symptoms appear uniformly on leaf/pod area. However, the results of the present study clearly demonstrated equal efficiency and accuracy of both, detached pod and germinated seed dip methods as similar disease reactions were obtained on test cultures using both methods.

As discussed above, SCAR marker *SY20* linked to gene, *Co-4* is reported to be used successfully to select gene-positive progenies in different bean breeding programmes throughout the world (Queiroz et al. 2004 and Vallejo and Kelly 2001). As far accuracy and efficiency of markers is concerned, the present study has clearly indicated the cent per cent accuracy and efficiency of SCAR markers *SY20* in selecting the gene, *Co-4* as indicated by the validation of imparted resistance in all 16 progenies. This is easily understandable in case of marker *SY20* that is known to be tightly linked with the gene *Co-4* gene has been developed from OPY20_{830C} RAPD marker at a distance of 0.0 ± 1.1 cM (Queiroz et al. (2004) and therefore is also expected to be equally efficient in indirect selection for the gene, *Co-4*.

In French bean pod shape is gaining more importance as an important agro-morphological attribute because of its role in post harvest processing. Straight pods are preferred more for easy handling and transport. Hence four *Co-4* gene-positive progenies exhibiting straighter pods can be likely used as donor of traits in addition to resistance gene, *Co-4*. BC₂F₄ progenies AKTO 4, AKTO 5, AKTO 7 and AKTO 43 of cross were identified as having better elite background along with the target gene can be potentially used as donor of various traits in addition to bean anthracnose resistance gene, *Co-4* as the likelihood of such parents contributing poor traits would be far less than the original donors and associated linkage drag can also be minimized.

Conclusion

The present study revealed the possibility of hybridisation and clearly validated the use of SCAR marker *SY20* for foreground selection in backcross breeding and tracking the genes in segregating populations of beans in selecting the gene *Co-4*. Comparable/better performance for some agro-horticultural traits revealed elite background of the identified stable homozygous derivatives (BC₂F₄) for gene, *Co-4* indicating success of backcross programme undertaken in the current study. The identified derivatives of cross can be further advanced using backcrossing to further improve the background and/ used as donors of resistance genes in the future bean breeding programmes.

Declarations

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Author contribution Bansuli formulated the initial data and RK Kapila corrected and wore the final manuscript.

Conflict of Interest All the authors declare that they have no conflict of interest in the publication.

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Tables

Table 1 Number of seeds harvested from BC₂F₂ gene-positive plant, seeds sown, number of seeds germinated and segregation status in each BC₂F₃ progeny

Progeny number	Number of seeds harvested from BC ₂ F ₂ gene-positive plant	Number of seeds sown	Number of seeds germinated	Seed Germination (%)	Marker status (830 bp band for Arka Komal × TO cross combination)		Segregation status of the progeny
					Number of gene-positive BC ₂ F ₃ plants	Number of gene-negative BC ₂ F ₃ plants	
Progeny 1	13	10	10	100	8	2	Segregating
Progeny 2	34	10	10	100	6	4	Segregating
Progeny 3	22	10	10	100	8	2	Segregating
Progeny 4	33	10	10	100	10	0	Non segregating
Progeny 5	32	10	10	100	10	0	Non segregating
Progeny 6	43	10	10	100	9	1	Segregating
Progeny 7	29	10	10	100	10	0	Non segregating
Progeny 8	37	10	10	100	10	0	Non segregating
Progeny 9	13	10	10	100	6	4	Segregating
Progeny 10	37	10	10	100	10	0	Non segregating
Progeny 11	17	10	10	100	7	3	Segregating
Progeny 12	55	10	10	100	7	3	Segregating
Progeny 13	18	10	10	100	9	1	Segregating
Progeny 14	18	10	9	90	8	1	Segregating
Progeny 15	44	10	9	90	8	1	Segregating
Progeny 16	37	10	10	100	7	3	Segregating
Progeny 17	17	10	10	100	7	3	Segregating
Progeny 18	24	10	10	100	8	2	Segregating
Progeny 19	18	10	10	100	10	0	Non segregating
Progeny 20	16	10	10	100	7	3	Segregating
Progeny 21	73	10	10	100	6	4	Segregating

Progeny 22	24	10	7	70	5	2	Segregating
Progeny 23	63	10	8	80	7	1	Segregating
Progeny 24	19	10	10	100	10	0	Non segregating
Progeny 25	24	10	8	80	4	4	Segregating
Progeny 26	26	10	9	90	8	1	Segregating
Progeny 27	16	10	9	90	7	2	Segregating
Progeny 28	16	10	10	100	10	0	Non segregating
Progeny 29	12	9	9	100	9	0	Non segregating
Progeny 30	30	10	7	70	4	3	Segregating
Progeny 31	13	10	8	80	7	1	Segregating
Progeny 32	38	10	8	80	7	1	Segregating
Progeny 33	25	10	10	100	10	0	Non segregating
Progeny 34	30	10	8	80	5	3	Segregating
Progeny 35	26	10	10	100	10	0	Non segregating
Progeny 36	46	10	9	90	8	1	Segregating
Progeny 37	22	10	8	80	6	2	Segregating
Progeny 38	16	10	9	90	7	2	Segregating
Progeny 39	16	10	7	70	6	1	Segregating
Progeny 40	40	10	10	100	10	0	Non segregating
Progeny 41	21	10	8	80	6	2	Segregating
Progeny 42	18	10	10	100	10	0	Non segregating
Progeny 43	24	10	10	100	10	0	Non segregating
Progeny 44	16	10	7	70	6	1	Segregating
Progeny 45	21	10	10	100	10	0	Non segregating
Progeny 46	30	10	10	100	10	0	Non segregating

Total Progenies = 46	1259	457	427	93.4	364	63
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Table 2 Disease score and reaction of 16 selected BC₂F₃ homozygous lines for *Co-4* gene against race 3 of *Colletotrichum lindemuthianum* using detached pod and germinated seed dip methods

Homozygous lines/ rows no.	Homozygous lines	Disease score		Disease reaction
		Detached pod method	Germinated seed dip method	
Row 4	AKTO 4	0	0	Resistant
Row 5	AKTO 5	0	0	Resistant
Row 7	AKTO 7	0	0	Resistant
Row 8	AKTO 8	0	0	Resistant
Row 10	AKTO 10	0	0	Resistant
Row 19	AKTO 19	0	0	Resistant
Row 24	AKTO 24	0	0	Resistant
Row 28	AKTO 28	0	0	Resistant
Row 29	AKTO 29	0	0	Resistant
Row 33	AKTO 33	0	0	Resistant
Row 36	AKTO 36	0	0	Resistant
Row 40	AKTO 40	0	0	Resistant
Row 42	AKTO 42	0	0	Resistant
Row 43	AKTO 43	0	0	Resistant
Row 45	AKTO 45	0	0	Resistant
Row 46	AKTO 46	0	0	Resistant
Control				
Arka Komal		5	5	Susceptible
TO		0	0	Resistant

Table 3 Characteristics of the parents and 16 *Co-4* gene-positive BC₂F₄ progenies for 8 qualitative agro-horticultural traits

Parents/ BC ₂ F ₄ progenies	Growth habit	Pod shape	Pod colour at picking stage	Stem colour	Seed coat pattern	Seed coat colour	Presence of colour on around of hilum	Brilliance of seed
AKTO 4	Bush type	Slightly curved	Green	Green	Mottled	Greyish white	Present	Medium
AKTO 5	Bush type	Moderately curved	Green	Green	Mottled	Greyish white to dark brown	Present	Medium
AKTO 7	Bush type	Moderately curved/ curved	Green	Green	Mottled	Greyish white to beige	Present	Medium
AKTO 8	Bush type	Slightly curved	Green	Green	Mottled	Greyish white to beige	Present	Medium
AKTO 10	Bush type	Slightly curved	Green	Green	Mottled	Greyish white to brown	Present	Medium
AKTO 19	Bush type	Straight	Green	Green	Mottled	Greyish white	Present	Medium
AKTO 24	Bush type	Slightly curved	Green	Green	Mottled	Greyish white to light beige	Present	Medium
AKTO 28	Bush type	Straight	Green	Green	Mottled	Greyish white to light beige	Present	Medium
AKTO 29	Bush type	Slightly curved	Green	Green	Mottled	Greyish white to beige	Present	Shiny
AKTO 33	Bush type	Straight	Green	Green	Mottled	Greyish white to Golden brown	Present	Medium
AKTO 36	Bush type	Straight	Green	Green	Mottled	Greyish white to Golden brown	Present	Shiny
AKTO 40	Bush type	Slightly curved	Green	Green	Mottled	Greyish white to Golden brown	Present	Shiny
AKTO 42	Bush type	Slightly curved	Green	Green	Mottled	Greyish white to dark brown	Present	Shiny
AKTO 43	Bush type	Slightly curved	Green	Green	Mottled	Greyish white to beige	Present	Shiny
AKTO 45	Bush type	Slightly curved	Green	Green	Mottled	Greyish white to pinkish brown	Present	Medium
AKTO 46	Bush type	Slightly curved	Green	Green	Mottled	Greyish white to	Present	Medium

light
brown

Parents								
Arka Komal	Bush type	Straight/ Slightly curved/ Moderately curved	Green	Green	Absent	Greyish white to brown	Present	Shiny
TO	Intermediate trailing	Straight/ Slightly curved	Green	Green	Mottled	Greyish white to beige	Present	Medium

Table 4 Analysis of variance for nine quantitative agro-horticultural traits in *Co-4* gene-positive backcross progenies

Source		Days to flowering Initiation	Days to 50 per cent flowering	Plant height at 50 per cent flowering (cm)	Branches /plant	Pod bearing nodes/plant	Pods/Node	Pod length (cm)	Pods/plant	Green pod yield/plant (g)
d.f										
M _r	2	9.40	5.68	52.06	0.17	0.72	0.37	1.62	5.56	67.04
M _g	17	17.83*	18.20*	372.73*	1.14*	2.90*	0.74*	5.77*	21.81*	484.24*
M _e	34	2.44	2.51	6.70	0.33	0.43	0.16	0.63	2.03	32.34
Cal F		7.30	7.24	55.65	3.46	6.76	4.76	9.16	10.76	14.96
SE(m±)		0.90	0.92	1.49	0.33	0.38	0.23	0.46	0.82	3.28
SE(d±)		1.28	1.30	2.11	0.47	0.53	0.32	0.65	1.16	4.64
CD		2.60	2.64	4.31	0.96	1.09	0.66	1.32	2.37	9.48
CV		4.10	3.89	6.07	16.64	13.01	15.18	6.14	14.38	12.78

* Significant at $P \leq 0.05$; Tab value of F at 17, 34 df = 1.93

Table 5 Performance of the parents and 16 *Co-4* gene-positive BC₂F₄ progenies for 9 quantitative agro-horticultural traits

Table 6 Promising *Co-4* gene-positive backcross progenies identified for 9 agro horticultural traits

<i>Co-4</i> gene-positive BC ₂ F ₄ progenies	Days to flowering Initiation	Days to 50 per cent flowering	Plant height at 50 per cent flowering (cm)	Branches /plant	Pod bearing nodes/plant	Pods/ Node	Pod length (cm)	Pods/plant	Green pod yield/plant(g)
AKTO 4	42.33	44.67	46.67	3.50	6.17	2.33	13.17	10.50	45.83
AKTO 5	37.50	39.00	35.17	3.00	5.33	2.67	13.60	13.67	57.50
AKTO 7	40.83	43.00	53.00	3.83	4.33	3.50	13.22	12.33	65.00
AKTO 8	37.00	39.67	41.83	3.67	4.50	3.50	11.65	12.17	53.33
AKTO 10	34.67	37.83	41.83	4.00	4.33	2.33	12.97	5.33	28.33
AKTO 19	35.67	40.33	41.33	3.33	4.17	2.67	13.85	8.33	44.17
AKTO 24	36.33	38.00	42.33	3.50	4.17	2.83	11.88	8.67	35.83
AKTO 28	36.67	39.33	40.50	2.50	4.33	2.17	13.43	7.33	31.67
AKTO 29	38.83	40.67	38.67	3.67	5.33	2.50	13.08	10.17	50.00
AKTO 33	39.67	43.33	40.67	3.33	4.67	2.17	13.20	11.00	37.50
AKTO 36	42.17	45.17	33.67	4.00	4.67	2.17	11.23	6.17	33.33
AKTO 40	38.83	41.50	36.33	4.00	5.83	2.67	12.98	12.33	53.33
AKTO 42	35.50	38.00	31.00	3.17	3.83	2.67	11.37	7.67	31.67
AKTO 43	37.00	39.67	50.50	3.00	8.00	3.33	14.20	14.67	61.67
AKTO 45	39.00	41.17	30.83	3.83	5.00	2.67	13.33	7.83	36.67
AKTO 46	38.50	40.67	37.17	3.33	5.67	2.50	13.98	7.33	32.50
Arka Komal	34.83	37.00	45.83	4.50	4.83	2.50	15.83	10.17	67.67
TO	41.50	44.00	80.17	1.83	5.33	1.50	9.53	12.50	35.00
SE(m±)	0.90	0.92	1.49	0.33	0.38	0.23	0.46	0.82	3.28
CD	2.60	2.64	4.31	0.96	1.09	0.66	1.32	2.37	9.48

Trait	Better parent (BP)	Progenies at par with elite parent (Four Best)	Progenies superior than elite parent (All)
Days to flowering initiation	Arka Komal	AKTO 10, AKTO 19 AKTO 24, AKTO 42	-
Days to 50 per cent flowering	Arka Komal	AKTO 5, AKTO 10 AKTO 24, AKTO 42	-
Plant height at 50 per cent flowering (cm)	Arka Komal	AKTO 4, AKTO 8 AKTO 10, AKTO 24	-
Branches/plant	Arka Komal	AKTO 7, AKTO 10 AKTO 36, AKTO 40	-
Pod bearing nodes/ plant	TO	AKTO 5, AKTO 29 AKTO 40, AKTO 46	AKTO 4, AKTO 43
Pods/node	Arka Komal	AKTO 5, AKTO 19 AKTO 24, AKTO 43	AKTO 7, AKTO 8 AKTO 43
Pod length(cm)	Arka Komal	-	-
Pods/plant	TO	AKTO 7, AKTO 8 AKTO 33, AKTO 40	AKTO 5, AKTO 43
Green pod yield/ plant (g)	Arka Komal	AKTO 7, AKTO 43	-

Figures

Figure 1

Agarose gel showing presence/absence of amplification products (830 bp bands) of SCAR marker, *SY20* in the parents and F_1 progenies (The first lane from left is molecular weight marker (100 bp ladder), P1= susceptible parent (Arka Komal), P2= resistant parent (TO), 1-11 are F_1 (Arka Komal $\times$ TO) hybrid pod progenies)

Figure 2

Agarose gel showing presence/absence of amplification products (830 bp bands) of SCAR marker, *SY20* in the parents and BC_1F_1 progenies (The first lane from left is molecular weight marker (100 bp ladder), P_1 = susceptible parent (Arka Komal), P_2 = resistant parent (TO), 1-3 are BC_1F_1 {Arka Komal $\times$ F_1 (Arka Komal $\times$ TO)} backcross pod progenies)

Figure 3

Agarose gel showing presence/absence of amplification products (830 bp bands) of SCAR marker, *SY20* in the parents and BC_2F_1 progenies (The first lane from left is molecular weight marker (100 bp ladder), P_1 = susceptible parent (Arka Komal), P_2 = resistant parent (TO), 1-6 are BC_2F_1 [Arka Komal $\times$ BC_1F_1 {Arka Komal $\times$ F_1 (Arka Komal $\times$ TU)}] backcross pod progenies)

Figure 4

Agarose gel showing presence/absence of amplification products (830 bp bands) of SCAR marker, *SY20* in the parents and BC_2F_2 plants (The first lane from left is molecular weight marker (100 bp ladder), P_1 = susceptible parent (Arka Komal), P_2 = resistant parent (TO) and 1-68 are BC_2F_2 hybrid pod progenies)

Figure 5

Agarose gel showing presence/absence of amplification products (830 bp bands) of SCAR marker, SY20 in the parents and 1-158 BC_2F_3 plants (The first lane from left is molecular weight marker (100 bp ladder), P1 = susceptible parent (Arka Komal), P2 = resistant parent (TO) and 1-158 are BC_2F_3 plants of Co-4 gene containing rows. The rows presented in red arrow are heterozygous and rows presented in yellow arrow are homozygous lines as all the plants in the row were gene-positive (showed lack of segregation)).

Figure 6

Agarose gel showing presence/absence of amplification products (830 bp bands) of SCAR marker, SY20 in the parents and 159-318 BC₂F₃ plants (*The first lane from left is molecular weight marker (100 bp ladder), P1 = susceptible parent (Arka Komal), P2 = resistant parent (TO) and 159-318 are BC₂F₃ plants of Co-4 gene containing rows. The rows presented in red arrow are heterozygous and rows presented in yellow arrow are homozygous lines as all the plants in the row were gene-positive (showed lack of segregation)*).

Figure 7

Agarose gel showing presence/absence of amplification products (830 bp bands) of SCAR marker, SY20 in the parents and 319-427 BC₂F₃ plants (*The first lane from left is molecular weight marker (100 bp ladder), P1 = susceptible parent (Arka Komal), P2 = resistant parent (TO) and 319-427 are BC₂F₃ plants of Co-4 gene containing rows. The rows presented in red arrow are heterozygous and rows presented in yellow arrow are homozygous lines as all the plants in the row were gene-positive (showed lack of segregation)*)).

Figure 8

Screening of BC₂F₃ progenies and parents against pathogen, *Colletotrichum lindemuthianum* race-3 using detached pod method **a)** Disease symptoms on susceptible parent 'Arka Komal' ; **b)** Healthy pods of resistant parent 'TO'; **c)** Row-1: AKTO- 4, 5, 7, 8, 10; Row-2: AKTO- 19, 24, 28, 29; Row-3: AKTO- 33, 36, 40, 42; Row-4: AKTO- 43, 45, 46

Figure 9

Screening of parents against pathogen, *Colletotrichum lindemuthianum* race-3 by germinated seed dip method **a)** Healthy leaves and stems of resistant parent 'TO'; **b)** Disease symptoms on susceptible parent 'Arka Komal'; **c & d)** Healthy leaves and stems of 16 homozygous lines for *Co-4* gene