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RESEARCH ARTICLE

Inheritance of flowering time in chickpea (*Cicer arietinum* L.) under cold stress conditions

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Abstract

Flowering is the primary characteristic of spermatophytes, which includes key crop plants like chickpea. The duration of flowering in plants with indeterminate growth is directly related to the yield. Early flowering in chickpea extends the reproductive phase, resulting in increased productivity. The experiment was carried out at the Department of Genetics and Plant Breeding, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, during the rabi season. The current investigation involved the crossing of the ICC-16349 (donor and cold-tolerant parent) with the GPF-2 (recipient and cold-susceptible parent) to produce a total of 80 potential hybrids. Screening of parents was conducted using 51 Simple Sequence Repeat (SSR) markers, of which marker TA-180 showed polymorphism. An evaluation was performed under field conditions to investigate the genetics of time to flowering of F_2 progeny plants resulting from the cross of chickpea genotypes GPF-2 (which flowers late under cold stress) and ICC-16349 (which flowers early under cold stress). In order to analyse the F_2 data and to assess the goodness of fit, the chi-square test (χ^2) was applied. During the experiment, TA-180 was used to screen 80 potential hybrids. The results showed that 34 of these hybrids (42.5 %) were confirmed to be actual hybrids. The F_2 generation of plants exposed to cold stress exhibited a segregation pattern, with a ratio of 3:1 between late and early flowering plants. This indicates that the trait of late flowering is under monogenic control that suppresses the trait of early flowering. The current work has the potential to aid in the creation of effective breeding strategies for the production of chickpea cultivars that exhibit early flowering under cold conditions. This would result in improved yields for chickpea crops grown in winter in Northern India.

Keywords: chickpea; *Cicer* sp.; flowering time; genetic control; inheritance; SSR; true hybrids

Introduction

The chickpea, (*Cicer arietinum* L.) (2n = 2x = 16), is a significant legume crop that originated during 7000 BC in Turkey (1). Chickpea is cultivated conventionally in semi-arid regions of India, Pakistan and the Middle East. Two main regions where chickpea diversity is concentrated are the Mediterranean area and South-West Asia (2). Ethiopia, on the other hand, is identified as a secondary center whereas South-Eastern Turkey and Northern Syria are believed to be its primary centre of origin (3). In general, there are 2 categories of chickpea: the Kabuli chickpea, which has larger seeds and the desi chickpea, which has smaller seeds (4). Chickpea is rich in protein, carbohydrates and minerals, particularly for individuals following a vegetarian diet. Its amino acid profile is composed of significant quantities of necessary amino acids except sulphur-containing amino acids. Its' mainly composed of high starch accompanied by a good amount of dietary fibre, simple sugars such as sucrose, glucose and oligosaccharides. Additionally, it serves as a rich reservoir of diverse vitamins such as thiamine, riboflavin, folate, niacin and a precursor to vitamin-A. When consumed alongside other cereals and legumes, it offers numerous health advantages. Several advantageous effects include safeguarding against cardiovascular diseases, digestive problems, type-2 diabetes and certain cancers. Therefore, it is a significant leguminous crop that offers a great range of nutritional and health advantages.

India, accounting for around 60 % of global production, holds the title of being the highest consumer as well as producer of chickpea (5). Nevertheless, despite being the primary producer of chickpeas, India continues to rely on imports from other countries. Low productivity of chickpea in India may be attributed to the widespread presence of abiotic and biotic stress factors, as well as the utilisation of low-yielding cultivars. Regarding abiotic stresses, temperature stresses are thought to be of utmost significance. Chickpea cultivation in northern regions of India is significantly impacted by cold temperatures, which adversely influence the crops' development, growth and total output (6, 7). Cold stress can manifest at any phase of plant development; yet, the magnitude of yield reduction escalates when the stress occurs during the flowering period. Cold stress during flowering leads to yield losses by causing flower abortion. Cold stress during flowering disrupts pollen

formation and ultimately causes pollen sterility, which is the primary reason for flower abortion. The absence of distinct physical characteristics for confirming hybridity in chickpea species, which is attributed to limited genetic diversity, along with a scarcity of markers, poses a significant challenge in identifying genuine hybrids in chickpea (8).

As a result, there is a possibility of misclassifying fake hybrids as true hybrids, which can lead to mistakes in breeding projects and a waste of money and effort. Currently, there is no published data regarding the degree to which bogus hybrids are used in chickpea improvement programmes. DNA-based markers are the preferred choice for determining the hybridity. Being codominant, highly specific to the target locus, SSR markers are the desirable DNA -based markers for chickpea (9). Simple sequence repeats are extensively employed in the investigation of genetic studies, as well as genome mapping, diversity and marker-assisted selection (10). The majority of significant legumes in India, such as chickpea, suffer from a scarcity of genetic resources, as very few SSR markers have been documented till now. Simple sequence repeat markers, which exhibit codominance, may identify alleles from both male and female parents and as a result, they are well-suited for distinguishing real hybrids from those that have self-fertilised.

The process of flowering has a direct impact on the output of crops. This crop frequently encounters short growing seasons due to cold stress (11). The character of early flowering is regarded as a crucial element in the development and production of pods before the onset of abiotic stressors. The duration that chickpea plants take to flower has a crucial role in determining their yield, particularly when they are exposed to unfavourable environmental circumstances (12). The blooming period in chickpea is influenced by altitude, latitude, sowing date and season (13). The duration for chickpea to start blooming is determined by the combination of photoperiod and temperature (14). The flowering time in certain chickpea genotypes was affected by photo period as well as temperature (15). In another study, it was examined the transmission of flowering time in chickpea was examined by a pair of genes (16).

The existence of a prominent gene (Efl-1/efl-1) along with other polygenes that influence the duration of flowering in chickpea is already validated (17). Validated the correlation between the primary gene responsible for the duration till blooming and the sensitivity to the length of daylight (Ppd/ppd) in chickpea (18). It is already determined that the primary genes accountable for early flowering (ppd and efl-1) were present at the same locus, although there was insufficient conclusive evidence. Research has demonstrated that quantitative data and identified a quantitative trait locus (QTL) associated with the number of days to 50 % flower initiation in chickpea (19). Another study concluded that the differentiation between a major gene and a QTL was arbitrary (20). Research indicates that a clearly characterised QTL might be considered as a major gene. Efficient timing management for flowering in chickpea is considered an important aspect of the chickpea breeding programme, particularly in cold stress conditions that negatively impact flowering and pod development, leading to reduced yield. Understanding the time to bloom in chickpea is necessary to design cultivars that are well-suited to cooler settings in northern India.

Materials and Methods

Plant material

For the current study, one cold-tolerant parent (ICC-16349) and other cold susceptible parent (GPF-2) were chosen for crossbreeding. The donor parent, ICC-16349, possessed cold tolerance, whereas the recipient parent, GPF-2, exhibited cold susceptibility. The F_1 generation resulting from the cross between GPF-2 and ICC-16349 was determined using SSRs. The second filial generation (F_2) of each first filial generation (F_1) plant was cultivated individually.

Confirmation of hybridity

The hybridisation process involved the crossing of two parents, namely ICC-16349 as the donor and GPF-2 as the recipient. The CTAB technique was applied for parental DNA extraction and an amplification pattern was generated using a set of 51 SSR markers, as listed in Table 1 (21–23). Of the total 51 SSR primers, TA-180 was able to depict genetic variation between the parents by comparison of the band size. The F_1 individuals carrying alleles from both of the parents were classified as true hybrids, distinguishing them from potential hybrids.

Experimental conditions

The experiment was carried out at the Genetics and Plant Breeding Department, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, during the rabi season (2018–19). The place is situated in the mid-hills of Himachal Pradesh (zone-II) and has high rainfall of 2500 mm, making it a humid sub-temperate environment. The highest temperature fluctuated between 7.0 and 29 °C, while the minimum temperature varied between 0.5 and 15.5 °C. The range of relative humidity fluctuated between 24 and 94 %, while the duration of sunshine ranged from 0 to 10 hr/day. The evaporation rate ranged from 0.4 to 5.4 mm/day.

Observations recorded

The phenotyping of F_2 plants derived from the cross between GPF-2 and ICC-16349 was carried out in field circumstances. The data on days until flower initiation was recorded to evaluate the plants' cold stress tolerance. The plants that bloomed simultaneously with the cold-resistant parent (ICC-16349) were classified as early-flowering, while the remaining plants were categorised as late-flowering, with their flowering period aligning with the cold-sensitive parent (GPF-2).

Statistical analysis

For testing the goodness of fit of the genetic ratio, Chi square test (χ^2) was applied to F_2 data. The following equation was used for calculating the χ^2 value (24):

$$x^2 = \sum_i \frac{(O_i - E_i)^2}{E_i} \qquad \text{(Eqn. 1)}$$

where χ^2 represents the chi-square value, O_i represents the observed frequency and E_i represents the expected frequency. The difference was regarded as significant between the expected and the observed values if the calculated χ^2value was higher than the tabulated value at (n-1) degrees of freedom (df).

Table 1. Description of SSR markers used for hybridity confirmation in the present study (*Polymorphic primer)

Sr No.	Primer name	Forward (5'-3')	Reverse	T_m (°C)
1	TA8	AAAATTTGCACCCACAAAATATG	CTGAAAATTATGGCAGGGAAAC	55.00
2	TA203	ATAAAGGTTTGATCCCCATT	TGTGCATTCAGATACATGCT	55.00
3	TR43	AGGACGAAACTATTCAAGGTAAGTAGA	AATTGAGATGGTATTAAATGGATAACG	55.00
4	TA30	TCATTAAAATTCTATTGTCCTGTCCTT	ATCGTTTTTCTAAACTAAATTGTGCAT	55.00
5	TA113	TCTGCAAAAACTATTACGTTAATACCA	TTGTGTGTAATGGATTGAGTATCTCTT	55.00
6	TA59	ATCTAAAGAGAAATCAAAATTGTCGAA	GCAAATGTGAAGCATGTATAGATAAAG	55.00
7	TA28	TAATTGATCATACTCTCACTATCTGCC	TGGGAATGAATATATTTTTGAAGTAAA	55.00
8	TA2	AAATGGAAGAAGAATAAAAACGAAAC	TTCCATTCTTTATTATCCATATCACTACA	55.00
9	TA146	CTAAGTTTAATATGTTAGTCCTTAAATTAT	ACGAACGCAACATTAATTTTATATT	55.00
10	TA72	GAAAGATTTAAAAGATTTTCCACGTTA	TTAGAAGCATATTGTTGGGATAAGAGT	55.00
11	TA116	AATTCAATGACGAATTTTTATAAGGG	AAAAAGAAAAGGGAAAAGTAGGTTTTA	55.00
12	TA130	TCTTTCTTTGCTTCCAATGT	GTAAATCCCACGAGAAATCAA	55.00
13	TR20	ACCTGCTTGTTTAGCACAAT	CCGCATAGCAATTTATCTTC	55.50
14	NCPGR209	ATTGTTTGTTGGAGTGATGG	CACGGTTTCATTGTCTTGTT	55.00
15	TA22	TCTCCAACCCTTTAGATTGA	TCGTGTTTACTGAATGTGGA	55.00
16	TA80	CGAATTTTTACATCCGTAATG	AATCAATCCATTTTGCATTC	55.00
17	TA176	ATTTGGCTTAAACCCTCTTC	TTTATGCTTCCTCTTCTTCG	55.00
18	TR44	TTAATATTCAAAAACTCTCTTGTGCAAT	TTTACAACAGCGCTTGTATTTAGTAAG	55.00
19	TR35	ACTTTGGTTTAACATTTTCGGTAGTTA	AGTATCAACGTCATGTGTAACTCGTAT	55.00
20	TR1	CGTATGATTTTGCCGTCTAT	ACCTCAAGTTCTCCGAAAGT	55.00
21	TA180*	CATCGTGAATATTGAAGGGT	CGGTAAATAAGTTTCCCTCC	55.00
22	TA14	TGACTTGCTATTTAGGGAACA	TGGCTAAAGACAATTAAAGTT	55.00
23	TA78	CGGTAAATAAGTTTCCCTCC	CATCGTGAATATTGAAGGGT	55.00
24	TA64	ATATATCGTAACTCATTAATCATCCGC	AAATTGTTGTCATCAAATGGAAAATA	55.00
25	NCPGR264	TGGGAATCTTGTTGGTTCTT	TGAAAGGAGATGGAAAAAGC	57.10
26	TS43	AAGTTTGGTCATAACACACATTCAATA	TAAATTCACAAACTCAATTTATTGGC	55.00
27	TA5	ATCATTTCAATTTCCTCAACTATGAAT	TCGTTAACACGTAATTTCAAGTAAAGAT	55.00
28	NCPGR263	CAAGGATGAATGTGTGTGTG	CATAGTATCCTCGGTTTCCC	55.50
29	NCPGR136	GGACTGAGTGAGTTCGTCTT	GTATCCTCGGTTTCCCTATC	54.00
30	NCPGR117	GAACTTCTTCAATCTCACGG	CTAGCACGATGAAAGGATTC	54.50
31	NCPGR247	CAATGATTGGTTCTCTCCTC	GGTTTGACTAAAATATGGCG	54.50
32	NCPGR281	GCAATGATTGGTTCTCTCCT	GTGGAATTCTTTAGGGTTTGAC	56.50
33	NCPGR231	AACCTCCGTCCACACATTTC	GGTCGAAGCCATTGTTTTGT	59.40
34	NCPGR224	TGGAATTAGTTGATGTGACAA	ATTTCCCGTGTCTTTGAGAT	59.20
35	NCPGR214	ATTTCCCGTGTCTTTGAGAT	GGAATTAGTTGATGTGACAATG	54.50
36	NCPGR127	CATAATGCAAGGGCAATTAG	CTCTTATCTTCATGTTGCCG	55.50
37	NCPGR111	AATAACTCCATTTGGCTTGA	GCGGTAATTACACAATACAGG	54.50
38	NCPGR142	TAACTCCATTTGGCTTGAGA	TAACCTTATATGGTAGGCGG	54.50
39	NCPGR252	TTGCCCTGAGGAATACATTA	GGTTGTTGAAGGCATAACTG	54.30
40	NCPGR255	TCAGTGGTATTGAGACATCG	CCATCTTCAAAAGTGAACCT	54.00
41	TA25	AGTTTAATTGGCTGGTTCTAAGATAAC	AGGATGATCTTTAATAAATCAGAATGA	55.00
42	TA42	ATATCGAAATAAATAACAACAGGATGG	TAGTTGATACTTGGATGATAACCAAAA	55.00
43	GA 11	GTTGAGCAACAAAGCCACAA	TTCTTGTCTGGTTGTGTGAGC	55.00
44	TS83	AAAAATCAGAGCCAACCAAAAA	AAGTAGGAGGCTAAATTATGGAAAAGT	55.00
45	TA96	TGTTTTGGAGAAGAGTGATTC	TGTGCATGCAAATTCTTACT	55.00
46	TA37	ACTTACATGAATTATCTTTCTTGGTCC	CGTATTCAAATAATCTTTCATCAGTCA	55.00
47	TA27	GATAAAATCATTATTGGGTGTCCTTT	TTCAAATAATCTTTCATCAGTCAAATG	55.00
48	NCPGR254	GCCTTTTTCAATTTCTCTCA	CCCAAAGAAGACAAAACAAC	54.50
49	NCPGR261	GATTGTGTGGCAAAATCCAT	ACTCTCAGGTTGCTGTTCTGA	58.90
50	NCPGR146	AACGTGAAATTCCACCACTA	GAGTCGATTTCGTGTTGATT	55.40
51	TA96	TGTTTTGGAGAAGAGTGATTC	TGTGCATGCAAATTCTTACT	55.00

Results and Discussion

The germplasm line 'ICC-16349', which is tolerant to cold stress, was chosen as the donor, while the cold susceptible parent 'GPF-2' was utilised as the recipient in the hybridisation process. Authentic hybrids were verified by the use of molecular markers (SSRs) and thereafter used for raising the F_2 generation. A total of 80 putative hybrid seeds were produced from parental crosses. Of the total 51 SSR primers, TA-180 was able to detect genetic variation between the parents. Therefore, TA-180 was utilised to verify the hybrid nature of the potential hybrids (Fig. 1). Among the 80 potential hybrids examined, only 34 had amplification of both the alleles associated with each parent used. This indicates that these 34 hybrids (42.5 %) were confirmed to be genuine, whereas the remaining 57.5 % were determined to be fake hybrids. The study proposed that DNA-based markers should be utilised in chickpea breeding programmes to verify hybridity and prevent the inclusion of self-pollinated plants in the crop improvement programme. The study also revealed that chickpea breeding incorporates a substantial proportion (>50 %) of false hybrids in crop improvement programmes, thus undermining the goal of enhancing chickpea quality. Several researchers have undertaken similar investigations on hybridity confirmation. The utilisation of SSR markers has already been employed to verify the hybrid nature of chickpea (25). Thirteen markers were able to distinguish both parents, Super Annigeri-1 and BS 72C2, were discovered. However, the marker ICCM0299 was capable of identifying hybridity in the F_1 generation and hence, it was utilised to confirm the hybridity status. In similar studies, genotyping of common bean was conducted using twenty-4 SSRs. Among the 342 F_1S resulting from 21 distinct parental crosses, a total of 325 (82.91 %) were verified to be genuine hybrids (26). Research indicates that out of the 25 SSRs, SSR-21 and SSR-22 exhibited parental polymorphism (27). The existence of F_1 hybrids (both intra-specific and interspecific) in the Mungbean crop that possess salt tolerance has already been demonstrated (28). The latter was achieved by utilising certain SSR markers that are associated with particular traits. Fifteen gene-specific SSRs were used to assess the hybridity of sixteen different

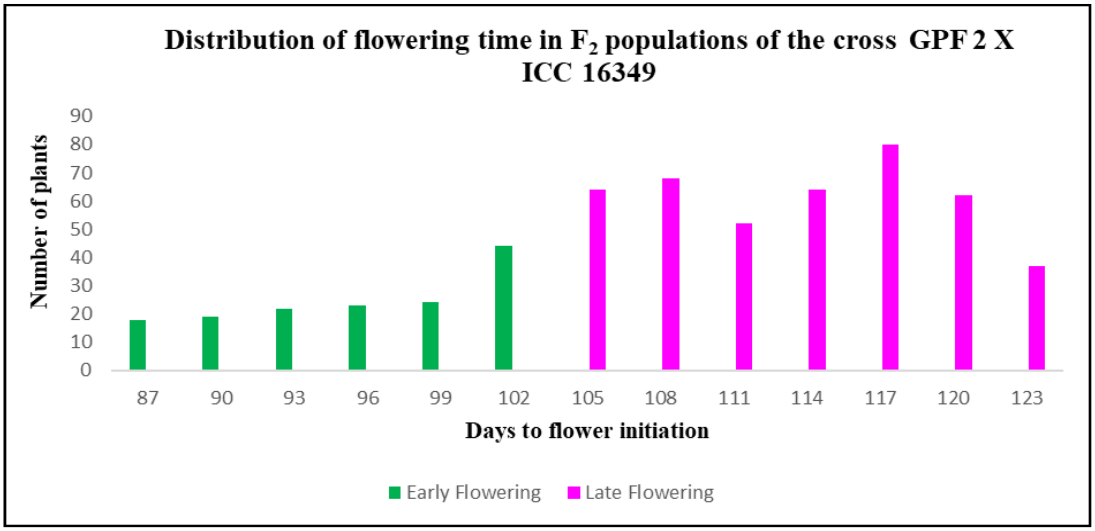

Fig. 1. Distribution of flowering time in F_2 populations of the cross GPF 2 × ICC 16349.

populations resulting from crossings between salt-susceptible and salt-tolerant lines. Polymorphism was observed only between the parents when using two primers, namely SSR3435 and SSR4041. In crops like maize, the SSR markers have been utilised for assessing genetic purity and similar work is conducted in rice (29, 30)

To ensure good crop varietal development programmes, sustaining the genetic production of genuine hybrid seed is essential. The utilisation of DNA markers provides clear benefits compared to biochemical and morphological indicators. Morphological markers and biochemical markers are significantly impacted by environmental influences and are unable to characterise related genotypes effectively, pertaining to their restricted polymorphism (30). DNA markers effectively address the limitations of biochemical and morphological markers, making them valuable tools for hybrid identification. The data about the time it took for the plants to initiate flowering showed that the F_2 progeny plants were divided into two groups: those that flowered early and those that flowered late (Table 2). Out of a total of 577 F_2 plants, 150 plants exhibited early blooming, whose flowering period coincided with that of the cold-tolerant parent (ICC-16349). The remaining 427 plants flowered late, corresponding to the flowering time of the cold-susceptible parent (GPF-2). Distribution of flowering time in F_2 populations of the cross GPF 2 × ICC 16349 is shown in Fig. 2. Chi-square test (χ^2) was used to assess the fit of the time to flowering data (Table 3) (31). The data demonstrated that the F_2 ratio of 3:1 (dominant: recessive) was consistent, indicating that the late flowering trait in chickpea was under a single gene exhibiting dominance. Several conclusions were drawn from different studies carried out to study the effect of cold stress on flowering in chickpea. Flowering is a critical time as chickpeas are more susceptible to cold stress during this stage (32, 33). Cold stress hindered microsporogenesis and pollen development (34). The cold stress sensitivity of flowers varied depending on the stage of the bloom, with younger flowers being more susceptible. Chickpea yield is negatively affected by cold stress due to delayed flowering and flower abortion (35). In another study, it was found that cooling stress negatively impacted the gynoecium by decreasing the number of fertilised ovules (36). Various research has shown that chilling stress negatively impacts the number of flowers in chickpea (37, 38). The study conducted on the time to flowering time inheritance indicated that two main genes, along with other polygenes, controlled the timing of flowering. The research also found that late flowering was monogenic control, which dominated early flowering (39).

Conclusion

Chickpeas exhibit indeterminate growth, meaning that the length of their reproductive phase can be prolonged by introducing the early flowering characteristic. Accelerated blooming has the potential to increase seed production in chickpea, particularly when faced with cold stress conditions that adversely affect flowering and pod development. The current study found that time of flowering in chickpea is largely under monogenic control, as also suggested. This trait can be easily introduced into a desirable cultivar by implementing breeding methods such as backcrossing or single-seed descent and can be used to develop early flowering in chickpea. Exploring mutation breeding may involve considering the potential conversion of a dominant trait into a recessive one. Further advancements in chickpea could be attained by integrating the early blooming characteristic into already well-suited cultivars, resulting in improved earliness and yield. The study also found that SSRs are dependable molecular markers for confirming hybridity. Additionally, it was shown that crossing chickpea plants could result in a significant number of self-pollinated plants, which may be mistakenly identified as hybrids. The study also showed that potential hybrids need to be verified using SSR markers in order to accurately identify true hybrids and avoid any mistakes in including false hybrids in the breeding programme.

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Authors' contributions

ST conceptualized the study and prepared the original draft of the manuscript. SS and K critically reviewed the manuscript and carried out necessary corrections and revisions. LM, PK and AS performed the data analysis and were responsible for formatting the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

Table 2. Segregation of F_2 plants obtained from cross ICC163499 (cold-tolerant) × GPF2 (cold-sensitive) of chickpea for early and late flowering

Sr. No.	Parents/F_1	Early flowering plants	Late flowering plants	Total (No.)	Flowering (No.)	Non-flowering (No.)
1	ICC16349*	1,2,3,4,6,7,8,9,10,11,12,13,14,15 ,16,17,19,22,23,24,25,26,27	5,18,20,21	27	23	4
2	GPF2*	-	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21	21	0	21
3	15S	2,3	1	3	2	1
4	16S	1,2,7,18,21, 25,28,30,31,34,44	3,4,5,6,8,9,10,11,12,13,14,15,16,17,19,20,22,23,24,26, 27,29,32,33,35,36,37,38,39,40,41,42,43,45	45	11	33
5	22S	7,16,19,25,26,27,30	1,2,3,4,5,6,8,9,10,11,12,13,14,15,17,18,20,21,22,23,24, 28,29	30	7	23
6	23S	5,12,13,17	1,2,3,4,6,7,8,9,10,11,14,15,16,18,19,20	20	4	16
7	31S	1,4,8,9,13,14,20,22,24	2,3,5,6,7,10,11,12,15,16,17,18,19,21,23,25,26,27,28,29 ,30	30	9	21
8	24S	3,4	1,2,5,6,7,8	8	2	6
9	26S	2,8,9,12,14,17	1,3,4,5,6,7,10,11,13,15,16,18,19	19	6	13
10	12S	2,8,11	1,3,4,5,6,7,9,10,12,13	13	3	10
11	11S	5,7,9	1,2,3,4,6,8,10,11	11	3	8
12	20S	1,5,13	2,3,4,6,7,8,9,10,11,12,14,15,16	16	3	13
13	13S	2,4,10,18,19,22	1,3,5,6,7,8,9,11,12,13,14,15,16,17,20,21	22	6	16
14	18S	3,8,9,12	1,2,4,5,6,7,10,11,13,14	14	4	10
15	38S	1,6,8,10,12,15,22,26	2,3,4,5,7,9,11,13,14,16,17,18,19,20,21,23,24,25,27,28, 29,30,31	31	8	23
16	17S	3,5,7,8,10	1,2,4,6,9,11,12,13,14,15,16,17,18,19,20	20	5	15
17	35S	1,3,6,8,14,23	2,4,5,7,9,10,11,12,13,15,16,17,18,19,20,21,22,24,25	25	6	19
18	4S	2,5,7	1,3,4,6,8,9,10	10	3	7
19	GPF2*	1	2,3,4,5,6,7,8,9,10,11,12,13,14	14	1	13
20	ICC16349*	1,2,3,4,5,6,9	7,8	9	7	2
21	29S	4,6,9,11	1,2,3,5,7,8,10,12,13,14,15,16,17	17	4	13
22	28S	2,7,9,12,16,22,26	1,3,4,5,6,8,10,11,13,14,15,17,18,19,20,21,23,24,25,27	27	7	20
23	37S	-	1	1	0	1
24	1S	1,3,6,7,9	2,4,5,7,8,10,11,12,13,14,15,16	16	5	11
25	19S	5,6,16,18,19,21,25	1,2,3,4,7,8,9,10,11,12,13,14,15,17,20,22,23,24,26	26	7	19
26	9S	1,7	2,3,4,5,6	7	2	5
27	34S	6,8,18,20	1,2,3,4,5,7,9,10,11,12,13,14,15,16,17,19,21	21	4	17
28	GPF2	-	1,2,3,4,5,6,7,8,9,10,11,12,13,14,1516,17,18,19,20,21	21	0	21
29	ICC	1,2,3,4,6,7,8,9,10,14,15,13	1,5,11,12,16	16	12	4
30	27S	4,8,9,11,18,28	1,2,3,5,6,7,10,12,13,14,15,16,17,19,20,21,22,23,24,25, 26,27	28	6	22
31	34P	1,10,12,14,15	2,3,4,5,6,7,8,9,11,13,16	16	5	11
32	33P	3,5	1,2,4,6,7,8	8	2	6
33	56P	2,9,11,12	1,3,4,5,6,7,8,10	12	4	8
34	28P	3,6	1,2,4,5,7	7	2	5
35	22P	1,5	2,3,4	5	2	3
36	2P	8,9,11,12	1,2,3,4,5,6,7,10	12	4	8
37	16P	4,8,9	1,2,3,5,6,7,10,11,12,13,14,15	15	3	12
38	57P	1,6,7,14	2,3,4,5,8,9,10,11,12,13,15,16	16	4	12
39	P-1-1**	2,3,9,14	1,4,5,6,7,8,10,11,12,13,15	15	4	11
40	P-2-1**	2,5,7	1,3,4,6,8,9,10	10	3	7
41	GPF2*	-	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,2 2	22	0	22
42	ICC16349*	2,3,4,6,7,8,9,12,13	1,5,10,11	13	9	4

*Parents **Reciprocal crosses

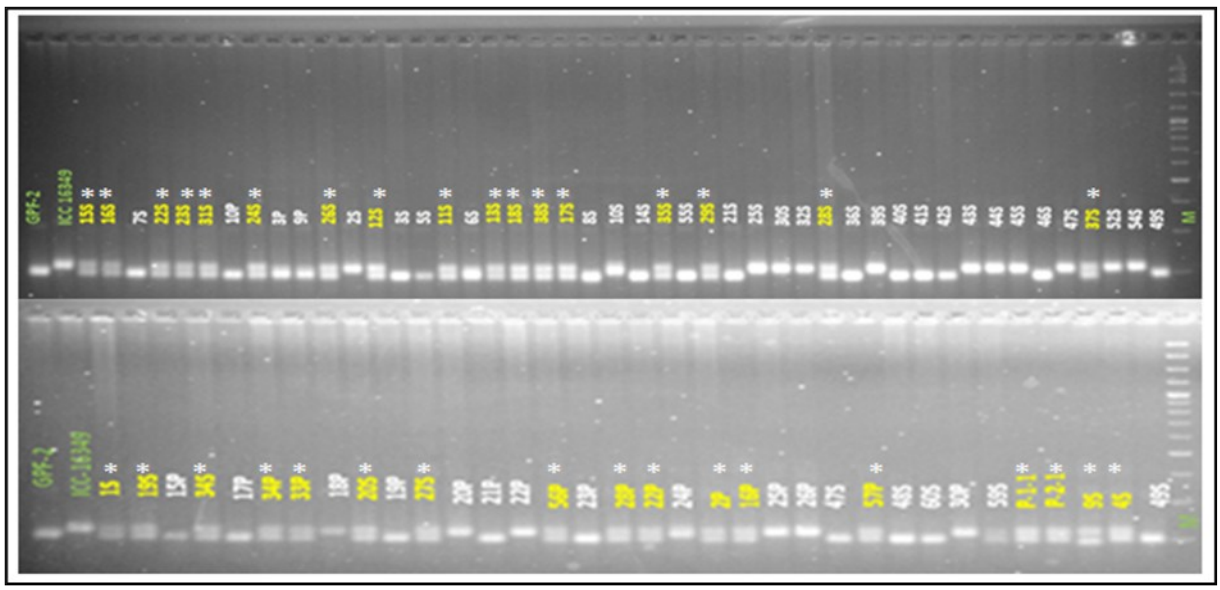

Fig. 2. Amplification pattern of parents (ICC-16349 and GPF-2) and putative hybrids as revealed by SSR marker TA 180. Names of parents and hybrids are given at the termini of lanes. M=100 bp DNA ladder, * = true hybrids

Table 3. Chi-square table pertaining to days to flower initiation for testing goodness of fit

Character	Observed	Expected	O-E	$(O-E)^2$	$(O-E)^2/E$
Early Flowering	150	144.25	5.75	33.0625	0.229202773
Late Flowering	427	432.75	5.75	33.0625	0.076400924
Total	577				χ^2=0.305603697

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