removal simulation. Figure 2 *b*, *c* shows the error in the processed image by the two schemes. Isotropic scheme is seen to give a better noise removal.

We also show a picture, taken from the web, Figure 3 *a*. A random noise is added to the picture, Figure 3 *b*. In order to run the noise-removal simulation, the integer pixel values, obtained using MATLAB, are converted to real values which are then divided by a factor 'FTOINT'. After the noise removal simulation, the real values are multiplied by FTOINT, and then converted to integer for plotting. Figure 3 *c*, *d* are pictures from the noise-removal simulation by the two schemes.

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Inheritance of wilt resistance in chickpea – A molecular marker analysis

The low productivity in chickpea is due to several biotic and abiotic factors. Among biotic factors, *Fusarium* wilt caused by *Fusarium oxysporum* f-sp *ciceris* is the major problem. A-1, the main variety under cultivation in Karnataka, being susceptible to wilt, the disease has spread to all the chickpea-growing regions of the state, severely affecting the productivity[1]. The screening of international germplasm has led to identification of stable sources of resistance to race 1 of *Fusarium oxysporum*, which is most prevalent in India. Genetic analysis has indicated that resistance to wilt race 1 is governed by (a) single gene[2–6], (b) two genes[7,8] and (c) three genes (H, H_2 and H_3)[9]. Partially recessive alleles in homozygous form at either of the first two loci and the dominant allele at the third locus delay wilting, but any two of these alleles together confer complete resistance[9].

Mayer *et al.*[10] developed an allele-specific associated primer (ASAP) CS-27_{700} which identifies already established locus (H_1) susceptible to race 1 of wilt pathogen. The marker was validated in a limited number of germplasm lines. However, there is a need to test this marker in different crosses and commonly used potential genotypes suitable for Indian conditions. In the present study an attempt has been made to validate the ASAP marker linked to H_1 in a cross between highly susceptible and resistant genotypes. An attempt was also made to understand the inheritance of wilt resistance coupled with segregation of associated marker.

JG-62, a highly susceptible, early-wilting genotype was crossed with WR-315, resistant to *Fusarium* wilt during *rabi* (2001) and advanced to F_5 generation by single seed descent (SSD) method using two seasons per year. The F_5 lines were tested for their reaction to wilt (race 1) in sick pots under net-house conditions[11]. A single spore isolate of pathogen *F. oxysporum* f-sp *ciceris* race 1 was obtained from International Crops Research Institute for Semi Arid Tropics (ICRISAT), Patancheru, and maintained on fresh potato dextrose agar (PDA). The inoculum was then increased in 100–150 g of corn-meal–sand (CMS) mixture in conical flasks and incubated for 21 days at room temperature[12]. The infested CMS was mixed thoroughly with an autoclaved soil mixture (clay loam, sand, FYM, 1 : 1 : 1 v/v) at (1 : 12 w/w) in earthern pots of 30 cm diameter. The seeds were surface-disinfected with 70% alcohol for 1 min and air-dried. The susceptible cultivar JG-62 was grown in all the pots (3–5 seeds/pot) and allowed to wilt. Seeds of the hundred F_5 genotypes were sown in these sick pots to study their wilt reaction. Three seeds/pot and two pots/genotype were maintained for each F_5 line. In each pot, one seed of JG-62 was also sown as reference. The pots were fertilized with 100 ml of Hoagland's solution every week[13]. The number of days taken from sowing to complete wilting was recorded for each genotype. Based on the number of days taken for complete wilt, the genotypes were classified as early-wilting (less than 25 days), late-wilting (25–55 days) and no wilting. Plants which remained healthy with no symptoms of wilting on the 55th day were considered as no wilting. The highly susceptible genotype JG-62 took less than 25 days for wilting in all the pots.

The hundred F_5 progenies, which were phenotyped for wilt reaction were used for validation of ASAP marker[10]. The DNA was extracted from all the progenies using CTAB method[14]. The DNA was used for polymerase chain reaction following the protocol of Mayer *et al.*[10]. The gels were scored for the presence or absence of specific marker (CS-27_{700}) linked to H-1 and tested for single locus goodness-of-fit for 1 : 1 segregation. The parental genotypes JG-62 and WR-315 showed polymorphism for the ASAP marker. Among the 100 F_5 progenies studied, 53 showed the presence of CS-27_{700} marker linked to H_1 and in the remaining the marker was absent (Figure 1). The goodness-of-fit for 1 : 1 segregation ratio showed single locus segregation of the linked marker (Table 1), thus supporting the contention that the parents differ at the locus linked to the marker.

Among 100 F_5 progenies studied under pot culture, 28 showed early-wilting, 43 showed late-wilting, while 29 showed no wilting (Table 2), segregating in the ratio

Table 1. Single locus goodness-of-fit for 1 : 1 ratio of presence and absence of ASAP marker (CS-27_{700}) in chickpea progenies

Locus	+	–	χ^2
CS-27_{700}	53	47	0.36

Table 2. Classification of F_5 progenies of chickpea for combination of wilt susceptibility and presence/absence of ASAP marker (CS-27_{700})

	+(Marker)	–(Marker)	Total
Early-wilting	28	0	28
Late-wilting	25	18	43
No wilting	0	29	29
Total	53	47	100

Table 3. Digenic phenotypic ratio of wilt reaction in F_5 generation of chickpea

EW ($H_1H_1H_2H_2$)	LW ($H_1H_1h_2h_2/h_1h_1H_2H_2$)	NW ($h_1h_1h_2h_2$)	χ^2
28	43	29	1.98

EW, Early-wilting; LW, Late-wilting; NW, No wilting.

Table 4. Goodness-of-fit for 1 : 1 : 1 : 1 genotypic segregation of wilt reaction in chickpea

EW ($H_1H_1H_2H_2$)	LW ($H_1H_1h_2h_2$)	LW ($h_1h_1H_2H_2$)	NW ($h_1h_1h_2h_2$)	χ^2
28	25	18	29	2.96

EW, Early-wilting; LW, Late-wilting; NW, No wilting.

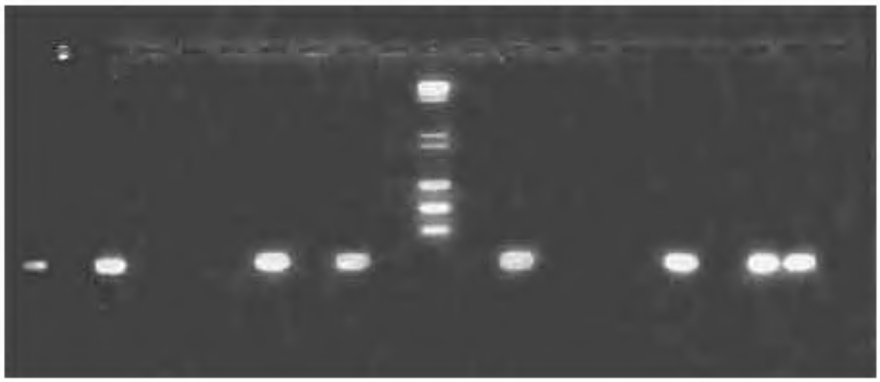

Figure 1. Allele-specific primer ($CS\text{-}27_{700}$) amplification of parental lines and F_5 progenies. Lane 1, JG-62; lane 2, WR-315; Lanes 3–10 and 12–21, F_5 progenies and lane 11, Molecular weight marker.

of 1 : 2 : 1 in F_5 generation, thus indicating that more than one gene is involved in resistance. Earlier studies indicated that dominant allele at that first two loci ($H_1H_1H_2H_2$) gives early-wilting and the recessive allele in homozygous form at any one of these two loci will give late-wilting ($h_1h_1H_2H_2$; $H_1H_1h_2h_2$), but recessive allele at both the loci confers complete resistance[9]. The parental genotypes chosen in the present study differed at the two loci, H_1 and H_2. It is expected to produce four classes of genotypes in the F_5 generation, which can be grouped into three phenotypic classes like early ($H_1H_1H_2H_2$), late ($H_1H_1h_2h_2/h_1h_1H_2H_2$) and no wilting ($h_1h_1h_2h_2$). The results indicate digenic inheritance of wilt resistance in this cross (Table 3).

In the present study, out of 52 progenies showing the presence of the marker, 28 were early-wilting. It is expected that all the early-wilting genotypes should have both H_1 and H_2. Consequently, the molecular marker linked to H_1. Among 43 late-wilting progenies, only 25 showed the marker. The late-wilting genotypes either have H_1 or H_2 in dominant conditions. Therefore, it is expected that only half of the late-wilting progenies have H_1 and the molecular marker, while the remaining half will have H_2 and absence of the molecular marker. The genotypes recessive at both the loci are resistant and do not contain the marker. Joint analysis of molecular marker and wilt reaction of the progenies, clearly supports the above expectations. The goodness-of-fit for digenic inheritance (1 : 1 : 1 : 1 ratio) of wilt resistance[8] in the present cross (Table 4) was further confirmed using ASAP marker linked to H_1. The results clearly support earlier results that there are two major genes for wilt resistance in chickpea[8]. However, the timing of early-wilting or late-wilting is subjected to a lot of variation. Such variation in timing of early or late-wilting could be due to the influence of several modifiers or QTLs with minor effect, which interact with the major genes and the environment. An RAPD marker linked to the H_2 locus of wilt resistance in chickpea has been identified in our laboratory (Thippeswamy *et al.*, under preparation). Attempts are being made to study the extent of effect of modifiers/minor genes on wilt resistance in different crosses using markers for both H_1 and H_2 loci.

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