Breeding for pod borer and wilt resistance in chickpea(*Cicer arietinum* L.)

Abstract

Chickpea(*Cicer arietinum* L.)is one of the important cultivating pulse crops globally and in India. Gram pod borer (*Helicoverpa armigera*) and *Fusarium* wilt (*Fusarium oxysporum*) are major pest and disease limiting the chickpea productivity. Pyramiding of these two biotic stress resistances in a single genotype is expected to increase crop productivity and reduces the usage of pesticides/fungicides, which favors the economic viability in the cultivation of this crop. Hence, it was planned to transfer the *cry2Aa* gene, from BS 72C2 event, to Super Annigeri-1(SA-1), the wilt resistant variety developed through pedigree method of breeding. The F_1s developed by SA-1XBS72C2 crosses were confirmed with *cry2Aa* gene-specific marker. These F_1s were advanced upto F_4 generation. The expression of the *cry2Aa* at the protein level through enzyme-linked immuno sorbent assay and insect bioassay were studied in F_4 generation.The average Cry2Aa protein expressed was 10.34 µg/gm whereas in control SA-1 it was zero. Forty F_4 plants were sown in wilt sick soil containing pots in green house. Other plants were sown in normal condition. Fifteen plants from wilt sick plots were PCR positive for both wilt disease and pod borer resistance. They also showed wilt resistance and were positive for qualitative ELISA test.

***Keyword**s*: Pedigree method,Bioassay, Backcrossing and Pyramiding

1. INTRODUCTION

Chickpea (*Cicer arietinum* L) known also as Bengal gram or Channa is the third most important pulse crop accounting for around 20 percent of world's pulse production. It is a low-cost source of vegetarian protein. It is cultivated in arid and semi-arid areas around the world. It is a self-pollinating diploid (2n=2x=16) with a relatively small genome (740Mb). It belongs to the family Fabaceae, subfamily Faboideae. The genus *Cicer* consists of 43 species with 9 annuals, 33 perennials and one unclassified (Van der Maesen, 1987).

It is extensively cultivated as winter crop. The crop is grown in nearly 57 countries with India, Australia, Myanmar, Ethiopia, Turkey, and Russia as the major producers (Merga and Haji 2019). India is the largest producer of chickpea accounting for about 68 per cent of the world chickpea production. In India it is cultivated in an area of 10.74 million hectares with a production of 13.54 million tones and productivity of 1261 kg per ha (PC Report, 2022-23). Karnataka ranks fifth in the cultivation of chickpea with an area of 7.12 lakh hectares with a production of 4.90 lakh tones and productivity of 689 kg per ha (PC Report, 2022-23).

Chickpea is considered as functional food as it contains 20-22% quality protein and is free from anti-nutritional factors compared to any other dry edible grain legumes (Williams and Singh, 1987). Despite its good qualities, chickpea area and production has not been improved much because of its vulnerability to various biotic and abiotic stresses. Currently chickpea is severely affected by half a dozen of pests and diseases. *Fusarium* wilt, caused by *Fusarium oxysporum* f. sp*. ciceris*, is the most important root disease of chickpea, particularly in the semiarid tropics where the chickpea-growing season is dry and warm (Nene *et al*., 1996).

Pod borer (*Helicoverpa armigera*) is the most important insect pest of chickpea globally. The insect is highly polyphagous and sources with high levels of resistance are not available in chickpea germplasm. Therefore, it has not been possible to breed varieties with adequate host resistance. Single larvae of the gram caterpillar *Helicoverpa* destroy 30-40 pods before its maturity (Bharati *et al.*,2015). An annual loss due to insect-pests is estimated to be 15 percent in chickpea. While the losses due to diseases like *Fusarium* wilt and *Ascochyta* blight are estimated around 600-750 thousand tonnes. Estimated loss in percentage due to Gram pod borer is 10-20% and *fusarium* wilt or root rot is 20-25%.

Keeping this in view, the availability of *Bt* event of chickpea from Assam Agricultural University, Jorhat and Wilt resistant variety, Super Annigeri-1 from UAS, Raichur, it is planned to transfer *cry 2Aa* genes to Super Annigeri-1.

2. MATERIAL AND METHODS

2.1 PLANT MATERIAL AND GENERATION OF F_4 POPULATION

Super Annigeri-1, *Fusarium* wilt disease resistant version of widely adapted Annigeri-1 but susceptible to pod borer (*Helicoverpa armigera*, Hubner) developed by University of Agricultural Sciences, Raichur and ICRISAT (Mannur *et al.,* 2019) was used as recipient parent. For pod borer resistance, chickpea *Bt* event, BS 72C2 (carrying *cry2Aa*gene), developed at Assam Agricultural University, Jorhat was used as donor parent. Artificial hybridization technique was used for generation of F_1 seeds from the cross between Super Annigeri-1 as recipient (♀) and *Bt cry2Aa* event (BS 172C2; ♂) as donor parents. True F_1s were identified using *cry2Aa* gene specific marker. F_1s were selfed to generate F_2-F_4 generation through pedigree method of breeding.

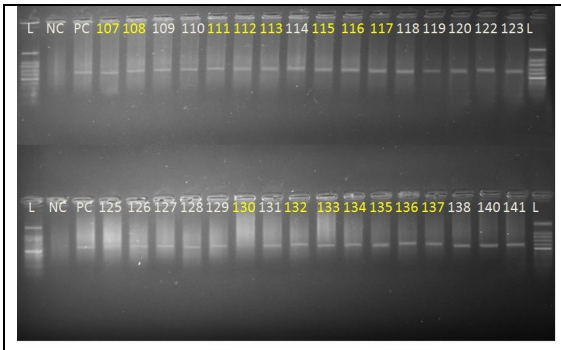
2.2 PCR ANALYSIS

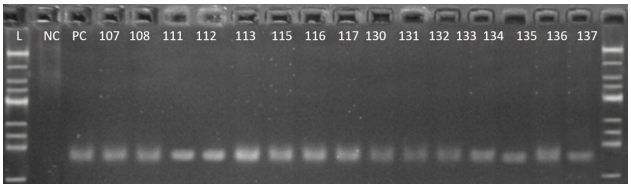
The gene specific primers(*cry 2Aa*)and TS 82 (Mannur *et al.,*2016;Varshney *et al.,*2014) wilt resistant markers were synthesized from Sigma Pvt Ltd. Polymerase Chain Reaction(PCR).PCR reaction mixture contained 1 µl of sample DNA, 2 µl of 2.5 mM,DNTPs, 0.5 µl of each forward and reverse primers,1 unit of Taq polymerase, 2 µl of 10X PCR buffer with MgCl2.Amplification cycle comprised of initial denaturation for 5 min at 94 ºC; 30 cycles of94 ºC for 45 sec, annealing depending on primers used for 50 sec and extension at 72 ºC for 1 min.;followed by a final extension at 72 ºC for 7 min in Master Cycler Gradient.

2.3 ENZYME-LINKED IMMUNOSORBENT ASSAY

Enzyme-Linked Immunosorbent Assay Cry2Aa expression in F_4 plants and transgenic donor and non-transgenic recipient parents was quantified through ELISA using Cry2Aa Qualitative and QuantiPlate kits (EnviroLogix, United States) for detection and quantification of Cry2Aa protein were used. Qualitative kit plate works on principle of Enzyme-Linked Immuno Sorbent Assay (ELISA). Leaf tissue extracts were added to wells, pre-coated with Anti- Cry2Aa antibodies. A secondary Anti- Cry2Aa Antibody conjugate is added to the wells after adding plant samples. Substrate was added after a wash, to detect Cry2Aprotein in samples through a color reaction. In quantitative ELISA, Cry2Aa protein concentration was estimated after recording OD vales under ELISA reader at 450nm wavelength. The amount of *Cry2Aa* protein in each leaf tissue sample (µg /gm) was determined using the formula Concentration (ppm) x µg weight of sample x dilution factor/ 1000

2.4 BIOASSAY FOR DETERMINING TOXICITY TO *H. ARMIGERA*





Entomocidal activity of the toxin Cry2Aa polypeptide expressed in the tissues of the F_4 chickpea plants was assayed through a detached leaf feeding bioassay along with control plants, using the neonate larvae of *H. armigera*.About 200–250 mg of fresh leaf material was placed in plastic petri dishes on moist filter paper and 8 neonate larvae of insect were placed on it. The plates were sealed with parafilm to prevent desiccation and kept in the lab room at 26 $\pm 1^0$C, 16 h photoperiod and 70% relative humidity. Feeding was allowed for 4 days with one change of fresh leaves at alternate days and data were taken on mortality. Corrected mortality was calculated by formula Treated mortality (%) - control mortality (%)/Treated mortality X100

2.5 IDENTIFICATION OF WILT RESISTANT PLANTS

The F_4 plants positive for *cry2Aa* were used to check wilt resistance.TS 82 wilt resistant marker was used to identify wilt resistant plants. Forty plants were also raised in pots filled with wilt sick soil in transgenic green house.

***2.6 PER SE* PERFORMANCE OF F_4 PLANTS**

The plants carrying both the genes of interest were recorded for yield and yield attributing traits. Unpaired T test was used compare F_4 plants with control plants (SA-1).

3.RESULTS AND DISCUSSION

3.1 PCR ANALYSIS

All forty F_4 generation plants raised in pots filled with sick soil were PCR positive for *cry2Aa*.Out of this fifteen plants were laso PCR positive for wilt resistance gene. (Table 1.)They also showed wilt resistance in wilt sick pots (Fig.6). In other 270 plants which were sown in normal condition in transgenic green house, 190 plants positive for *cry2Aa.*Out of these 190 positive plants 62 plants were PCR positive for wilt resistance(Table 2.) The plant number positive for both traits are 62.

Fig1.F_4 plants in sick soil pot showing amplification for cry2Aa gene L-ladder (100bp), Negative Check (NC) :SA-1, Positive Check (PC) :BS72c2	Fig 2.F_4 plants in sick soil pot showing amplification for of wilt resistance gene(TS82) L-ladder (100bp),Negative Check (NC): JG-62(susceptible check),Positive Check (PC) :SA-1

(a) Table 1.PCR analysis of plants sown in wilt sick soil filled pots in transgenic green house

No of plants sown in sick soil (pot)	PCR for trait (gene)	Results
40 F_4plnts	Pod borer Resistance (*cry2Aa*)	40 positive
	Wilt Resistance (TS82)	15 positive

Table 2.PCR analysis of F_4 generation plants raised in transgenic green house

Sl no	Genotypes	No plants	*cry2Aa* positive plants	For both Wilt and *cry2Aa* positive plants	Wilt positive plants
1	F_4 Plants	273	190	62	62
2	SA-1	30	-	-	30

Fig 3.F_4 plants showing amplification for *cry2Aa* gene

L-ladder (100bp),NC-SA-1,PC-BS72c2

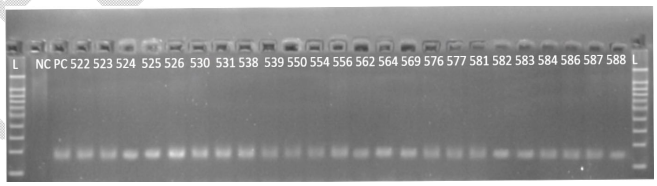


Fig 4.F_4 plants amplification for of wilt resistance gene (TS 82)

L-ladder (100bp),NC-JG-62(susceptible check),PC-SA-1

Fig.5 F_4 Plants

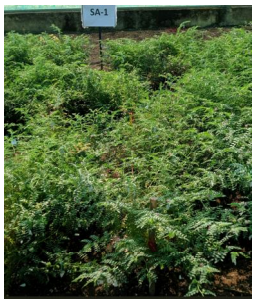


Fig.6 SA-1plants

Fig 7.F_4 plants sown on in pots containing sick soil

3.2 EXPRESSION OF CRY 2AA PROTEIN IN F_4 POPULATION AT TRANSLATIONAL LEVEL

Enzyme Linked Immuno-Sorbent Assay (ELISA) was carried out for qualitative (Fig.5) and quantitative (Fig.5) assessment of Cry2Aa protein in F_4 generation plants sown in transgenic green house (Fig 5-6), Cry2Aa protein expressed ranged from 10.01 to 10.68 µg/gm(Table.3).

Table 3.Quantitative estimation of Cry2Aa protein (µg/gm tissue) in F_4 generation

Sl No	Plant no	Cry2Aa protein µg/gm
1	F4-P-108	10.42
2	F4-P-111	10.17
3	F4-P-134	10.54
4	F4-P-116	10.49
5	F4 R-542	10.37
6	F4 R-549	10.49
7	F4 R-550	10.17
8	F4 R-562	10.63
9	F4 R-569	10.44
10	F4 R-611	10.20
11	F4 R-657	10.68
12	F4 R-677	10.48
13	F4R-684	10.51
14	F4 R-685	10.51
15	F4R-688	10.55
16	F4 R-691	10.01
17	F4 R-698	10.62
18	F4 R-702	10.48
19	F4 R-713	10.54
20	72C2	10.34
21	SA-1	0.0001
	Mean	10.34
	Variance	0.03

	SD	0.17

3.3 INSECT BIOASSAY (DETACHED LEAF ASSAY) IN F_4 GENERATION

In F_4 population insect bioassay (Fig.7) revealed that 78.21% to 95.62% corrected larval mortality (Table 4.) The plants numbered F4-P-136, F4 R-611, F4 R-677, F4-R-698 and Bt event BS-72C2 (positive control) recoded 95.62% larval mortality. The corrected larval mortality in negative control, is zero.

Table 4. Insect bioassay of F4 plants

Sl No	Plant no	Cumalative mortality%			Corrected mortality (%)
		24hr	48hr	72hr	
1	F4-P-108	0	45.8	91.6	91.24
2	F4-P-111	0	37.5	83.3	82.59
3	F4-P-134	0	45.8	91.6	91.24
4	F4-P-135	0	45.8	91.6	91.24
5	F4-P-136	0	45.8	95.8	95.62
6	F4-P-137	0	41.6	91.6	91.24
7	F4 R-542	0	41.6	91.6	91.24
8	F4-R-569	0	41.6	87.5	86.97
9	F4 R-611	0	45.8	95.8	95.62
10	F4-R-636	0	58.3	83.3	82.59
11	F4 R-677	0	45.8	95.8	95.62
12	F4R-684	0	66.6	91.6	91.24
13	F4-R-688	0	66.6	91.6	91.24
14	F4-R-691	0	41.6	79.1	78.21
15	F4-R-698	0	45.8	95.8	95.62
16	72C2	0	45.8	95.8	95.62
17	Control(SA-1)	0	0	0	0
	Variance				28.23
	SD				5.31

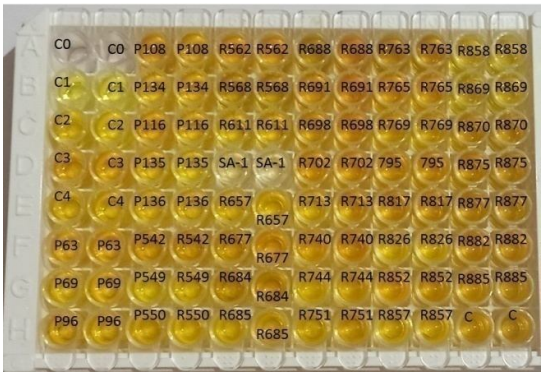


Fig.8 Qualitative ELISA for F_4 plants

Fig.9 Qualitative ELISA for F_4 plants, C-72C2

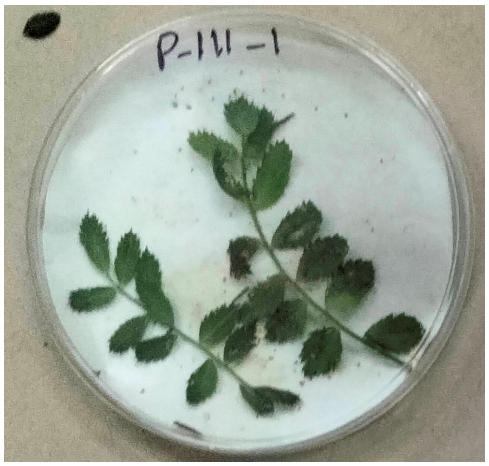


Fig.10 Insect bioassay for F_4 plants

3.4 IDENTIFICATION OF WILT RESISTANT PLANTS

The F_4 plants positive for *cry2Aa* were checked for wilt resistance using wilt resistance marker TS-82. Out of 230 positive plants 77 plants showed 182bp amplicon with TS 82 marker. The plants positive for both the traits are 77.

3.5 PER SE PERFORMANCE OF F_4 GENERATION PLANTS POSITIVE FOR BOTH WILT AND *CRY2AA* GENES

Plant height, number of primary and secondary branches, number of pods per plant and seed yield per plant were accounted. The height of the F_4 generation plants ranged from 36 to 95cm with mean value of 61.28 cm which is significantly higher than mean of SA-1 (38.5 cm). The F_4 plants numbered F4 R-538, F4 R-549, F4 R-550, F4 R-556, F4R-562, F4R-564, F4R-569, F4R-593, F4R-634, F4R-684 and F4R-684 were taller recorded above average height(61.28cm).The minimum number of secondary branches recorded were 3 while maximum numbers of branches recorded were 13 with average value of 6.59 in F_4 plants which is again significantly higher than SA-1 (4.29) .The number of pods recorded ranged from 27 to 295 with mean value 70.96 in F_4 plants which is again significantly higher than SA1 (39.27). The average yield per plant recorded was 15.24 gm per plant in F_4 generation whereas in control plants, it was significantly lower (10.53 gm).

Table 5.Yield and yield attributing traits in F4 plants and control plants

Sl No	Plant no	Height (cm)	No of primary branches	No of secondary branches	No of pods per plant	Yield/plant (gm)
1	F4 R-522	68.00	2.00	8.00	41.00	8.70
2	F4 R-523	70.50	1.00	4.00	65.00	9.50
3	F4 R-524	63.00	1.00	6.00	55.00	8.10
4	F4 R-525	82.00	2.00	6.00	29.00	8.07
5	F4 R-526	67.00	2.00	5.00	40.00	11.10

6	F4 R-530	52.00	2.00	5.00	45.00	8.10
7	F4 R-531	45.00	1.00	6.00	40.00	7.80
8	F4 R-538	81.00	2.00	6.00	70.00	17.10
9	F4 R-539	52.00	1.00	6.00	45.00	6.20
10	F4 R-543	42.10	1.00	8.00	60.00	9.90
11	F4 R-549	93.00	1.00	8.00	65.00	10.20
12	F4 R-550	88.00	2.00	10.00	58.00	9.50
13	F4 R-554	74.00	2.00	5.00	51.00	10.24
14	F4 R-556	86.00	2.00	7.00	82.00	13.10
15	F4 R-562	88.00	2.00	8.00	64.00	9.80
16	F4 R-564	78.00	1.00	6.00	92.00	21.20
17	F4 R-569	86.90	2.00	5.00	78.00	19.45
18	F4 R-575	53.00	2.00	6.00	65.00	8.10
19	F4 R-576	64.00	1.00	6.00	45.00	6.50
20	F4 R-577	55.30	2.00	8.00	42.00	8.20
21	F4 R-581	66.80	1.00	7.00	36.00	7.50
22	F4 R-582	49.00	2.00	9.00	45.00	5.71
23	F4 R-583	55.00	1.00	2.00	27.00	8.20
24	F4 R-584	63.00	1.00	2.00	33.00	5.92
25	F4 R-586	71.00	1.00	6.00	40.00	6.23
26	F4 R-587	66.00	1.00	7.00	60.00	7.10
27	F4 R-588	51.10	2.00	7.00	40.00	6.10
28	F4 R-589	59.00	4.00	7.00	34.00	5.80
29	F4 R-590	65.00	1.00	5.00	39.00	5.90
30	F4 R-593	77.00	1.00	7.00	57.00	15.50
31	F4 R-621	66.00	2.00	6.00	42.00	8.90
32	F4 R-634	77.10	1.00	6.00	83.00	23.20
33	F4 R-636	53.00	2.00	7.00	83.00	24.00
34	F4 R-644	57.80	1.00	6.00	58.00	8.30
35	F4 R-649	55.00	2.00	6.00	59.00	12.50
36	F4 R-652	51.00	2.00	5.00	41.00	8.50
37	F4 R-665	64.00	1.00	6.00	55.00	8.20
38	F4 R-667	65.60	1.00	5.00	54.00	13.10
39	F4 R-668	58.00	1.00	6.00	44.00	8.50
40	F4 R-683	45.00	2.00	6.00	60.00	9.10
41	F4 R-684	95.00	3.00	8.00	78.00	14.80
42	F4 R-685	71.40	2.00	9.00	59.00	13.25
43	F4 R-686	58.00	2.00	6.00	55.00	12.10
44	F4 R-687	71.00	1.00	5.00	70.00	13.80
45	F4 R-689	87.00	1.00	8.00	63.00	10.63
46	F4 R-706	70.00	2.00	2.00	70.00	15.10
47	F4 S-367	64.00	2.00	8.00	65.00	8.80
48	F4 S-371	38.00	2.00	7.00	68.00	10.50
49	F4 S-372	42.00	3.00	8.00	295.00	75.88
50	F4 S-373	36.00	1.00	6.00	45.00	11.50
51	F4 S-375	43.00	3.00	7.00	39.00	10.96

52	F4 S-377	42.00	3.00	6.00	148.00	31.20
53	F4 S-383	56.50	2.00	6.00	69.00	20.10
54	F4 S-387	44.50	3.00	8.00	119.00	33.06
55	F4 S-388	58.00	3.00	7.00	192.00	51.51
56	F4 S-390	43.00	3.00	6.00	116.00	30.52
57	F4 S-391	44.00	1.00	6.00	65.00	11.20
58	F4 S-392	46.00	3.00	11.00	95.00	29.60
59	F4 S-394	40.00	3.00	8.00	157.00	34.01
60	F4 S-400	45.00	2.00	8.00	70.00	10.42
61	F4 S-401	46.00	3.00	13.00	169.00	45.10
62	F4 S-402	65.00	3.00	9.00	171.00	42.21
63	Control -1	39.00	1.00	5.00	52.00	12.60
64	Control -2	48.00	2.00	5.00	54.00	12.10
65	Control -3	43.00	1.00	6.00	61.00	12.40
66	Control -4	37.00	2.00	4.00	41.00	11.80
67	Control -5	38.00	1.00	4.00	28.00	10.50
68	Control -6	32.00	2.00	3.00	28.00	10.40
69	Control -7	34.00	1.00	4.00	38.00	10.10
70	Control -8	33.00	1.00	3.00	33.00	9.30
71	Control -9	35.00	2.00	4.00	38.00	10.50
72	Control -10	36.00	2.00	4.00	38.00	10.50
73	Control -11	42.00	1.00	4.00	33.00	10.10
74	Control -12	44.00	2.00	3.00	34.00	9.60
75	Control -13	43.00	1.00	4.00	22.00	8.90
76	Control -14	39.00	2.00	4.00	22.10	8.80
77	Control -15	42.00	1.00	3.00	19.00	8.50
78	Control -16	38.00	2.00	5.00	40.00	11.50
79	Control -17	36.00	1.00	4.00	24.00	9.20
80	Control -18	37.00	1.00	5.00	23.00	9.10
81	Control -19	36.00	2.00	4.00	44.00	10.10
82	Control -20	38.00	1.00	3.00	50.00	11.90
83	Control -21	34.00	1.00	6.00	52.00	12.10
84	Control -22	36.00	2.00	5.00	35.00	9.90
85	Control -23	43.00	2.00	4.00	39.00	9.80
86	Control -24	44.00	1.00	4.00	55.00	11.60
87	Control -25	36.00	1.00	5.00	61.00	11.20
88	Control -26	37.00	1.00	5.00	68.00	12.50
89	Control -27	44.00	1.00	4.00	34.00	9.80
90	Control -28	33.00	1.00	5.00	40.00	10.20
91	Control -29	38.00	1.00	5.00	58.00	11.60
92	Control -30	40.00	2.00	4.00	35.00	9.50

Table 6. Unpaired T test for yield and yield attributing traits F_4 plants control plants

	n	Plant height(cm)	No of primary branches	No of secondary branches	No of pods per plant	Yield/plan (gm)
		Mean	Mean	Mean	Mean	Mean
F_4 plants	62	61.28	1.82	6.59	70.96	15.24
Control plants	30	38.50	1.40	4.20	39.97	10.53
't'stat		7.93	2.70	6.68	3.62	2.10
't' critical two tail		1.98	1.98	1.98	1.98	1.98

Table 7.Variance and standard deviation for yield and yield attributing traits F_4 plants

	Plant height(cm)	No of primary branches	No of secondary branches	No of pods per plant	Yield/plant (gm)
Mean	61.28	1.82	6.59	70.96	15.24
Variance	238.24	0.61	3.49	2094.62	164.74
Standard deviation	15.44	0.78	1.87	45.77	12.83

3.6 DISCUSSION

In efforts of introgression breeding for combining desirable traits, backcross breeding aims at adding desirable trait into recurrent parent lacking one or few desirable traits but a popular variety needs to restoration of all other many desirable traits of that variety. In pedigree method of breeding combining many traits to derive all together different variety. So in the present study the two diverse lines , one genotype containing *cry2Aa* gene (BS 72C2) imparting pod borer resistance from Assam Agricultural University, Jorhat, Assam and another genotype, Super Annigeri 1resistant to *Fusarium* wilt from University of Agricultural Sciences Raichur, Karnataka were used to generate F_4 generation plants. Since the generated F_4 genetic material is possessing transgene, *cry2Aa*, it was evaluated in the transgenic green house. The plants containing pod borer and wilt resistance plants were successfully detected in F_4 segregating population with PCR and it was also validated with their expression through ELISA test and insect bioassay studies. This is the first study of the introgression of two traits for biotic stress resistance. Following few studies are on transferring only cry gens for pod borer resistance from events to elite genotypes.

Kaur *et a*l.,(2019) reported conversion of elite but pod borer-susceptible commercial chickpea cultivars into resistant cultivars through introgression of *cry1A*c using marker-assisted backcross breeding. The chickpea cultivars (PBG7 and L552) were crossed with pod borer-resistant transgenic lines (BS 100B and BS 100E) carrying *cry1Ac* that led to the development of BC_1F_1, BC_1F_2, BC_1F_3, BC_2F_1, BC_2F_2, and BC_2F_3 populations

from three cross combinations. The average cry2Aa protein expressed was 10.34 µg/gm whereas in control SA-1 it was zero. The F_0 plants have exhibited upto 95.62%neonatal larval mortality. Similarly, Mehrotra *et al.,* (2011) observed expression of *Cry1Ac* toxins in transgenic chickpea in the range from 5 to 40 µg per gram of leaf tissue. Sanyal *et al.,*(2005) also estimated the *Cry1Ac* protein accumulation which showed extractable protein with maximum range between 14.50 to 23.5 µg per gram of leaf tissue in T0 and T1 transgenic chickpea plants. Smitha *et al*., (2022) reported The F_1 plants developed by SA-1 X BS 100B crosses were confirmed with *cry1Ac* gene-specific marker and polymorphic SSR marker (ICCM0299).The expression of the *cry1Ac* gene at the transcriptional level through reverse transcription polymerase chain reaction and at the protein level through enzyme-linked immuno sorbent assay was confirmed in F_1. BS 100B (donor parent), F_1 and F_2 plants, respectively recorded 21.47 µg, 20.43 µg and 15.31-21.17µg of *Cry1Ac* protein/g of leaf tissue in quantitative ELISA test that is enough to record pest resistance. Out of 230 *cry2Aa* positive F_4 plants 77 showed wilt resistance. Smitha *et al*., (2022) reported TS 82 marker was screened on the 30 F2 plants confirmed with *cry1Ac* gene and four plants wilt resistance TS-82 marker. In our study what is interesting is apart from generating genetic resource for pod borer and wilt resistance, they are also significantly superior in plant height, primary and secondary branches, number of pods and seed yield than SA 1 the commercial variety.

References

Ajinder Kaur, Urvashi Sharma, Sarvjeet Singh, Ravinder Singh, Yogesh Vikal,Satnam Singh, Palvi Malik1, Khushpreet Kaur, Inderjit Singh, Shayla Bindra,Bidyut Kumar Sarmah and Jagdeep Singh Sandhu.,2019,Introgressing cry1Ac for pod borer resistance in chickpea through marker-assisted backcross breeding

Bharti,Premlata., Singh S. P. N., and Nagendra, Kumar.,2015, Efficacy of some insecticides, bio products and their phototoxic effect against gram pod borer (*HelicoverpaarmigeraHub*.) in chickpea *Current Biotica* 9(3):247-255, 2015

Mannur,D,M.,Yeri,S,B.,Mahiboobsa,M.,Shankergouda,I.,ThudiMahendra,Varshney,R,K.*et al.,* 2016, Introgress of foc 4 QTL locus to develop “Super Annigeri-1”chickpeavariety resistant to *Fusarium wilt* through MABC.*VIII international Scientific Agriculture Symposium, “Agrosym2016”, 6-9 october 2016, Jahorina, BosniaandHerzegovina.Proceedings*1598 1603

Mannur, D. M., Anita, B., Mahendra, T., Murali, M. S., Manish, R., Yeri, S. B., Vijay, P. B., Jayalakshmi, S. K., Mallikarjun, B. P. and Varshney, R. K., 2019, Super Annigeri-1 and improved JG 74: two *Fusarium* wilt-resistant introgression lines developed using marker-assisted backcrossing approach in chickpea. *Mol. Breeding,* 39: 2.

Mehrotra, M., Singh, A. K., Sanyal, I., Altosaar, I. and Amla, D. V., 2011, Pyramiding of modified *cry1Ab* and *cry1Ac* genes of *Bacillus thurengiensis* in transgenic chickpea (*Cicer arietinum* L.) for improved resistance to pod borer insect *Helicoverpa armigera*, *Euphytica*, 182: 87-102

Merga, B., and Haji, J. (2019). Economic Importance of Chickpea: Production,Value, and World Trade. Cogent Food Agric. 5, 1615718. doi:10.1080/23311932. 2019.1615718

Nene, Y.L., Sheila, V.K., Sharma, S.B., 1996, A World List of Chickpea and Pigeonpea Pathogens, 5th edn. ICRISAT, Patancheru, India p 27.

Project coordinators report, 2022-23 all India coordinated research projects on Rabi pulses.p 26,33.

Sanyal,I.,Singh,A.K.,Kaushik,M.andAmla,D.V.2005*Agrobacterium*-mediated transformation of Chickpea (*Cicer arietinum*L.) with *Bacillus thuringiensis cry1Ac* gene for resistance against podborer insect *Helicoverpa armigera*.*Plant Science*168,1135-1146.

S. Smitha, N. V. Mohan Kumar, I. S. Katageri, M. D. Patil and S. Anjan,Gowda.,2022,Introgression of transgenic event in a molecular breeding variety for pyramiding resistance to *fusarium* wilts and pod borer in chickpea (*cicer arietinum* l.) .*Int.J.Curr.Microbiol.App.Sci*(2022) *11*(12): 91-102

Van der Maesen L J G.,1987, Origin, history and taxonomy of chickpea. In: Saxena M C, Singh R B (ed) *The Chickpea.* pp 11-34. CABI Publishing, Wallingford, UK.

Varshney,R.K.,Mohan,S.M.,Gaur,P.M.,Chamarthi,S.K.,Singh,V.K.and Samineni, S. *et al.,* 2014a. Marker-Assisted Backcrossing to introgress resistance to *Fusarium* wilt race 1 and *Ascochyta blight* inC 214, an elite cultivar of chickpea (*Cicerarietinum*L.).*The Pl. Genome*7,1-11.

Williams, P. C and Singh, U., 1987, Nutritional quality and the evaluation of quality in breeding programmes. In: Saxena M C and Singh K B (eds) *The Chickpea*. Pp 329– 90. CAB International, Wallingford.