Evaluating Bt cotton (*Gossypium hirsutum* L.) under different management practices

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To,

The controller of examinations,
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Faisalabad.

We, the supervisory committee, certify that the contents and form of thesis submitted by **Muhammad Faisal Bilal**, Regd. No. 2004-ag-2022, have been found satisfactory and recommend that it be processed for evaluation, by External Examiner(s) for the award of degree.

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DEDICATED TO

HOLY PROPHET MUHAMMAD
(Peace be upon him)
THE GREATEST SOCIAL REFORMER

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List of abbreviations

Abbreviation	Description
%	percent
cm	centimeter
cm^2	square centimeter
DAS	days after sowing
EC	electrical conductivity
Fig.	figure
g	gram
ha^{-1}	per hectare
kg	kilogram
m^{-2}	per square meter
N	nitrogen
P	phosphorus
K	potassium
t ha^{-1}	tonnes per hectare
NAWF	Nodes above white flower
NACB	Nodes above cracked boll
BCR	Benefit cost ratio
MRR	Marginal rate of return (%)
LSD	Least significant difference
NS	Non significant

Abstract

Removal of early fruiting branches with optimum/higher nitrogen and potassium dose can cause more source and no sink at early stages leading to delay in onset and progression of senescence in Bt cotton. The present investigations were carried out to find out interactive effect of square/fruiting branch removal in Bt cotton cultivar IR-3701 using different nitrogen and potassium doses. Two experiments were planted during 2011 and repeated during 2012 at the Agronomic Research Area, University of Agriculture Faisalabad. First experiment comprised of manual alteration of plant architecture i.e. F_1: no fruiting branch removal, F_2: removal of first fruiting branch, F_3: removal of first and second fruiting branch, F_4: removal of all squares (floral bud) from first fruiting branch, F_5: removal of all squares from first and second fruiting branch; and three nitrogen rates i.e. N_1: 175, N_2: 225 and N_3: 275 kg ha^{-1}. Removal of first and second fruiting branch and removal of all squares from first and second fruiting branch along with increasing nitrogen dose improved yield, yield components and quality of cotton as well as helped in delayed onset of senescence and in increasing Cry1Ac concentration in boll pericarp; but on the basis of marginal rate of return, removal of first and second fruiting branch with medium level of nitrogen application (225 kg N ha^{-1}) was the best treatment combination. In second experiment manual alteration of plant architecture (same treatments as in experiment 1) was combined with three potassium rates i.e. K_1:50, K_2: 100 and K_3: 150 kg K ha^{-1}. Results showed that removal of first and second fruiting branch and removal of all squares from these branches at higher level of potassium application (150 kg K ha^{-1}) improved yield, yield components and quality traits, reduced premature senescence and also improved Bt gene expression in late season by increased uptake of N and K in plant. Highest BCR as well as maximum MRR were obtained by supplying the crop with 150 kg K ha^{-1} and removing first and second fruiting branches at early stages.

INTRODUCTION CHAPTER-1

Cotton "white gold" or "the king of fiber" is the leading fibre crop world wide and is grown commercially in more than 50 countries (Smith, 1999). Cotton crop primarily grown for fiber and oil purpose (Oosterhuis, 2001). Pakistan is one of the largest cotton consuming and producing countries and one of the ancient homes of cotton cultivation. Cotton has played significant role in agriculture, employment, industrial development, financial stability and economic viability ever since the country attained the independence. Bt (*Bacillus thuringiensis*) cotton is rapidly dominating world cotton production due to tolerance to the insects (Pray *et al*., 2002). In 2011-12, cotton was grown on an area of 2835 thousand hectares with production of 13.6 million bales. The production was higher by 18.6% over the last year production (11.5 million bales); the increase in production is attributed to the cultivation of Bt cotton with average yield of 815 kg ha^{-1}. Cotton has 7.8% of value added in agriculture and 1.6% to GDP (Govt. of Pakistan, 2012). Among cotton producing countries China has highest productivity levels followed by USA, Uzbekistan, Pakistan and India (Khadi *et al*., 2010). In the world about 26.247 million metric tons of cotton are produced annually and main cotton contributing countries are USA, China, Pakistan, India, Uzbekistan, Australia, Turkey, Greece, Brazil and Egypt (Usha, 2010).

In 1996 Bt cotton was planted in the United States commercially (Hardee and Herzog, 1997). Bollgard cotton commonly known as Bt cotton was developed by Monsento as a new approach to control insect pests injury in cotton (Jenkins *et al*., 1997). Biological characteristics of Bt cotton cultivars vary from conventional cotton cultivars. Bt (transgenic) cotton varieties have a draw back of slow emergences but first true leaf appearance is early than conventional cotton varieties (Zhao *et al*., 2002). The main objective is to offer Bt cotton to farmers with more environmental safety and efficient insect pest control at lower expenditure (Benedict and Altman, 2001). Cry1Ac gene in Bt cotton cultivars (Perlack *et al*., 1991) that cause resistant to insects pests, which is an agronomic advantage (Perlack *et al*., 2001). Bt gene and expression of Bt toxin protein caused change in metabolic processes both in reproductive and vegetative growth in Bt cotton (Chen *et al*., 2002). Greenplate (1999) reported significant differences in Cry1Ac protein expression between six field sites, indicating environmental influences on

production or stability of the Cry1Ac protein. Cry1Ac concentrations in plants also showed no specific trend over time despite variation among sampling timings and tissue types, which indicated that environmental conditions may influence Cry1Ac protein expression (Greenplate *et al*., 2000). Often, Bt protein levels decline with time, but Wan *et al*. (2005) observed that levels rebounded late in the season. Different plant tissues, growth stages, and cultivars provide significant sources of variation in protein levels, but some cultivars were more variable than others (Rochester, 2006).

Removing early fruiting branches (REFB) increased lint yields more than 5 % (Dongmei *et al*., 2009). Total nitrogen, Cry1Ac protein, soluble protein and glutamic-pyruvic transaminase (GPT) activity in leaves were higher in early fruiting branches removal than control (Dongmei *et al*., 2009). Early season squares and fruiting branches removal increased root growth (Dumka *et al*., 2004) photosynthetic rate (Wells, 2001; Dumka *et al*., 2003) and Cry 1Ac expression in Bt cotton (Dongmei *et al*., 2009). Poor efficacy of Bt transgenic cotton against boll worms is mainly recognized by reduction of Cry1Ac protein in different parts (Benedict *et al*., 1996). In Bt cotton reduction in Cry1Ac protein was due to deficiency of nitrogen (Coviella *et al*., 2002) or with higher doses of nitrogen application (Pettigrew and Adamczyk, 2006); indicating a significant impact of nitrogen metabolism on Cry1Ac concentration in Bt cotton(Chen *et al*., 2005a), while removal of early flower bud could alter N metabolism (Deng *et al*., 1991). It is hypothesized that early fruiting branches removal might improve the Cry1Ac appearance in Bt cotton (Dongmei *et al*., 2009).

Among all nutrients nitrogen is required in larger amount and consistently for good cotton growth (Hou *et al*., 2007). Nitrogen application and its uptake efficiencies were influenced by numerous factors e.g field management, fertility status of soil and yield potential however, nitrogen can be applied in moderately low dose with more efficient manner than traditional way (Clawson *et al*., 2008; Kumbhar *et al*., 2008). Seed protein content increased with 50 kg ha^{-1} nitrogen application (Patil *et al*., 1997). In Bt cotton cultivars, at peak squaring and boll opening, total N contents were higher than their parents; N uptake in the leaf of Bt cotton improved after introduction of the Bt gene (Chen *et al*., 2005b). In dynamic crop like cotton extremes of nitrogen encouraged vegetative growth and delayed maturity that resulted in lower yield (McConnell *et al*., 1996). Opened bolls per plant were higher with application of 143

kg N ha^{-1} as compared to 95 kg N ha^{-1} (Sawan *et al*., 2006). Reduced auxins content in the leaves and stems were due to nitrogen deficiency (Anisimov and Bulatova, 1982). Increase in boll weight was observed with increased nitrogen application from 95 to 143 kg ha^{-1} (Sawan *et al*., 2006). Leaf photosynthetic rates increased from 11% to 29% with application of 157 kg N ha^{-1} (Cadena and Cothren, 1995). Nitrogen played vital role in formation of protein (Frink *et al*., 1999). Nitrogen and carbon is required for seed development (Patil *et al*., 1996). At early crop development stage due to deficiency of N ethylene was probably produced in response to N-deficiency stress which increased shedding of floral buds, flowers and bolls in cotton (Lege *et al*., 1997).

Next to nitrogen, potassium is the mineral element required in higher quantity by cotton (Oosterhuis *et al*., 2013). For optimal cotton growth 2 to 5% K is required on dry weight basis (Marschner, 1995). From agronomic point of view, deficiency and excess of potassium are environmentally and economically unproductive and have harmful impact on yield of cotton (Oosterhuis *et al*., 2013). Unnecessary K application in cotton enhanced plant height (Pettigrew and Meredith, 1997) and postponed maturity (Clement and Gwathmey, 2007; Gwathmey *et al*., 2009); while deficiency of K decreased lint percentage (Pettigrew *et al*., 1996), lint yield (Read *et al*., 2006), dry matter production (Rosolem *et al*., 2003), plant height (Zhao *et al*., 2001), internodal length, leaf area (Gerardeaux *et al*., 2010), seed & boll mass (Pettigrew *et al*., 1996) and nitrogen uptake(Pettigrew and Meredith, 1997). Potassium deficiency affects crop maturity by stopping reproductive growth prematurely (Pettigrew, 2003). Second to nitrogen on about 110 to 250 kg K ha^{-1} is required for growth of cotton and development of fiber (Hodges, 1992); 2 to 5 kg potassium ha^{-1} d^{-1} (Mullins and Burmester, 1990) with 50% potassium in the boll (Rimon, 1989) and 24% in the lint and seed (Mullins and Burmester, 2009).

Use of potassium for production of cotton has not been common however, cotton crop yield and quality, before cultivation of Bt cotton farmer mainly used organic manure to fulfill K requirement which maintained a moderate level of available K in soil (Dong *et al*., 2004). But with time, use of organic manure became limited and Bt varieties were found to be more sensitive to potassium deficiency than non Bt varieties (Zhang *et al*., 2007).

Potassium deficiency induces numerous disorders in cotton e.g. decreased leaf area index, plant biomass and photosynthesis, but enhances earliness (Hezhong *et al*., 2004). Obvious contributors to K deficiency may be adoption of modern Bt cotton varieties and low K supply (Hezhong *et al*., 2004). Although high yielding Bt cotton cultivars showed potassium deficiencies (Phipps *et al*., 2003) but extra potassium resulted more vegetative growth (Gwathmey, 2005) and delayed maturity (Gwathmey and Howard, 1998). Pettigrew *et al*. (2005) reported that deficiency of potassium caused earlier maturity of cotton. Potassium plays a vital role in plants resistance and tolerance to pathogens, storage process, unfavorable soil and climatic conditions, assimilate transport, nitrogen metabolism, phloem loading and controlling the water relationship in plants (Hezhong *et al*., 2004). Sufficient potassium is also needed for the efficient use of nitrogen (Varco and Fridgen, 2004). Symptoms of premature senescence spread in cotton crop as season progresses and defoliation occurred; this potassium related premature senescence was found in China (Zheng and Dai, 2000), USA (Oosterhuis, 2001) and Australia (Mott, 2003). Potassium perhaps induced its chief influences on disease through precise metabolic functions that modify compatibility associations of the host-parasite surroundings (Kafkafi *et al*., 2001).

Keeping in view the above facts, the present experiments were conducted

- To study the effect of removal of squares and/or fruiting branches under different N and K levels on growth, yield and quality of Bt cotton.
- To investigate, whether delayed fruiting initiation can help to over come pre mature senescence of Bt cotton.
- To study, whether the squares and/or fruiting branches removal with different N or K can alter the Bt gene expression in term of late season insect pests control
- To calculate economic feasibility of removal of squares or branches in cotton with different N and K applications

2.1 Discovery and development of Bt cotton

In 1901 Sigetane Ishiwata (Japanese scientist) isolated bacterium (*Bacillus thuringiensis*) from silkworm, Bombyx mori larvae (Ishiwata, 1901). In 1911 Ernst Berliner (German scientist) isolated rod shaped bacterium from diseased larvae of *Ephestia kahuniella* (Zell) found in Thuringia, Germany (Berliner, 1911). He named the bacterium as Berliner that is a soil bacterium (*Bacillus thuringiensis*) that produces insecticidal proteins during its sporulation. Edward Steinhaus in the 1950's at the University of California conducted further research on Bt and made biopesticides e.g. Thuricide® and Dipel® for commercial use (Steinhaus, 1951). With the progression in the field of genetic engineering the δ-endotoxin gene of *B. thuringiensis* became an attractive candidate gene that was transferred into plants. The Cry genes are responsible for production of δ-endotoxin crystal in *Bacillus thuringiensis* and this gene was then transferred in var. kurstaki cotton via. Agrobacterium with Ca MV 35 S promoter (Umbeck *et al*., 1987). Perlak *et al*. (1990) for the first time reported truncated forms of insect control protein genes of *B thuringiensis* var. karstaki strain HD-1 (Cry 1 Ab) and strain HD-73 (Cry 1 Ac) in cotton plants. Transgenic Bt-cotton plants expressed insect control protein and provided useful floral bud, flower and boll protection about 70 to 87% against Helicoverpa insect pest (Venugopal *et al*., 2002). In transgenic cotton Bt δ-endotoxin is produced when a lepidopteran pest eats square/flower/boll; this protein entered in gut system (having alkaline situation) and gets bound to specific receptor sites of alimentary canal after activation. The protoxin damages the wall of gut leads to paralysis and ultimately death(Tabashnik, 1994). In the world scenario, Bt-cotton offered high level of resistance against cotton bollworm (Shelton *et al*., 2002). Transgenic cotton showed great resistance against *H. armigera* (American cotton bollworm) both under laboratory and field conditions (Ghosh, 2001; Kranthi, 2002).

Square/Fruiting branch removal

Early season flowers removal might be favorable to mid season boll development. Its importance would depend on total yield however, flower losses by hand removal or insect pests

can draw many physiological and morphological responses including compensatory growth (Wells, 2001; Wilson *et al*., 2003).

Square removal and leaf cut treatments were applied on Bt cotton cultivars Sikang 1 and Sikang. Seed index and boll shell volume were reduced by leaf cut, but increased by square removal. Square removal reduced the insecticidal protein in boll shell as compared to leaf cut treatment. Square removal increased boll size and reduced Cyr1Ac protein content but leaf removal increased Bt toxin but decreased size of boll (Yonghui *et al*., 2009). In late developmental stage Bt insecticidal gene expression level was reduced due to the changes of metabolic characteristics during development (Xia *et al*., 2005). Before boll period N metabolism was strong and carbohydrate metabolism was weak, the struggle for N between vegetative growth and reproductive growth was weak (Guinn, 1986). Growth of floral bud, flower and synthesis of Bt protein was not affected by sufficient nitrogen supply (Chen *et al*., 2000).

Result of two years field trial on Bt cotton cultivar SCRC-showed that two basal fruiting branches removal at squaring increased leaf area, plant biomass, the plant height, lint yield (5.2 to 7.5 %), boll size (5.1 to 5.7 %), number of fruiting nodes, Cry1Ac protein in young leaves and Cry1Ac expression in term of more insects pest resistance and improved the fiber strength and micronarie compared with their control treatment (Dong *et al*., 2008). Removal of squares prolonged anthesis (Jones *et al*., 1996). Early season square removal increased root growth, and fruiting branch removal altered the spatial yield distribution (Bednarz and Roberts, 2001; Dumka *et al*., 2004). Dumka *et al*. (2003) reported that removal of early squares increased rate of photosynthesis rate. In cotton plant early square loss using growth regulator compensated on lower sympodia (Cook and Kennedy 2000), and higher nitrogen application has ability to improve this compensation potential (Malik *et al*., 1981).

In Bt cotton Bt gene expression, in the form of insecticidal proteins, caused in vegetative as well as reproductive growth by altering its metabolism (Chen *et al*., 2002: Tian *et al*., 2000). In Bt cotton insecticidal protein in leaf was strongly correlated with nitrate reductase, protease and glutamic-pyruvic transaminase activity (Chen *et al*., 2003). Prebloom square removal was applied to three cultivars of Bt cotton; yield increase was observed in the treatment where all squares were removed from the plant one week after squaring began. Only the treatment where

all squares were removed before bloom significantly reduced yield and caused delay in crop maturation. Moderate levels of square removal had little impact on overall lint production. Prebloom square loss, increased ability of the Bt cotton to insect pest management (Stewarta *et al*., 2001). High nitrogen application and square removal stimulated number of fruiting positions and main stem elongation per plant. Nitrogen application increased both number of nodes on main stem and mean internodal length whereas square removal increased only node numbers on main stem. Compensation following square removal was greater at higher nitrogen application than at lower nitrogen application (Malik *et al*., 1981).

Nitrogen Fertilization

Plant height, nodes per plant, sympodial branches, first sympodial position, opened bolls, seed index, lint percentage and seed cotton yield per plant were increased by increasing nitrogen levels (Emara and Gammaal, 2012). Deficiency of N in cotton which may induce premature senescence leading to potential yield loss (Girma *et al*., 2007).

Seed oil content was slightly decreased with an increase in the nitrogen level from 95.2 to 142.8 kg ha^{-1}, but seed oil yield had significantly increased (45.5 kg oil ha^{-1}), which is attributed to the significant increase in cotton seed yield (Sawan *et al*., 2007). In cotton crop yield increases were attributed to the truth that nitrogen was a chief nutrient in controlling new growth, thus influencing boll weight, boll development, and number of bolls per plant (Patil *et al*., 1996).

Improvement in growth and nutrient content in cotton plant was observed with 142.8 kg N ha^{-1} than with lower dose (Sawan *et al*., 2009). Application of fertilizer N is typically required to optimize lint yield of irrigated cotton (Jin *et al*., 1997). Increasing N from 0 to 120 kg ha^{-1} enhanced above ground biomass production (Perumai, 1999). While increasing N from 90 to 157 kg ha^{-1} did show no improvement in cotton yield (Boquet, 2005).

Nitrogen application at 150 and 200 kg ha^{-1} produced more bolls per plant, seeds per boll, average boll weight and seed cotton yield as compared to the lower rates (Khan and Dar, 2006). Optimum doses of nutrients particularly of nitrogen could increase seed cotton yield (Marsh *et al*., 2000). Boll weight increased by increase in application of nitrogen to cotton crop (Ram *et al*., 2001). Increased leaf photosynthetic rates (11% to 29%) were recorded with the application of 157 kg N ha^{-1} (Cadena and Cothren, 1995). At the reproductive stage low nitrogen supply

decreased leaf net photosynthetic rate, leaf area and chlorophyll content while enhanced fruit abscission in cotton plant (Zhao and Oosterhuis, 2000)

Lint percentage was not affected with increasing nitrogen from 45 to 134 kg ha^{-1} (Phipps *et al*., 1996). Fiber length, strength and micronaire increased by increasing nitrogen dose, but effect was too small while there was no effect on fiber uniformity (Sawan *et al*., 2006). Nitrogen levels did not exhibit significant effects on fiber quality traits except the lint percentage (Saleem *et al*., 2010c).

Cotton leaves from were collected from deficient, adequate, and excessive N fertility plots for Cry1Ac expression from nodes 8, 13 and 18. Leaf Cry1Ac protein expression significantly increased with increasing N fertilizer application. As the season progressed, symptoms of N deficiency (light green plants) or excess N (dark green plants) were observed in the deficient and excessive N rate treatments. Cry1Ac protein expression was significantly higher in the older leaves (node 8) than the younger leaves (node 18) (Rochester, 2006). The fifth uppermost cotton leaf from the terminal was sampled at flowering, mid-boll fill and 20% open boll. Leaf Cry1Ac protein expression was non-significant at all the levels of N application more over lint yield was not improved above 70 kg ha^{-1}N (Rochester, 2006).

An experiment showed that highest seed cotton yield was recorded in 240 kg N ha^{-1} x 100 kg K ha^{-1} treatment with an increase of 14% over the control. While the highest K uptake efficiency of 42% was recorded in 240 kg N ha^{-1} x 0 kg K ha^{-1} treatment. It was concluded that application of nitrogen increased potassium uptake when cotton crop was grown on moderate fertile land (Khalifa *et al*., 2012).

Potassium Fertilization:

Approximately 2.6% potassium is present in the earth's crust but deficiency of potassium is common on large farming areas (Aleman *et al*., 2011). Cotton farmers use more quantity of nitrogen (125 kg ha^{-1}) but potassium use is minor i.e 0.7 kg ha^{-1} (Reddy *et al*., 2000). The principal plant nutrient determining crop yield and quality is potassium (Pettigrew, 2008). Lower application of potassium fertilizer to cotton has serious threats; because cotton crop is more sensitive to potassium deficiency than many other crops because cotton root system is less impenetrable than other crops (Abdul-Malak and Mukrum, 1996). Adoption of Bt cotton

cultivars with fast fruiting, more boll, early maturity and high yielding appears to be more sensitive to potash limitation than non Bt cultivars (Wright, 1999). Bt cotton cultivars are more susceptible than non Bt cotton cultivars because Bt cultivars retained more early fruit (Wright, 1998). Bt cotton cultivars have less source and more sink than conventional cotton, which consequences in an unequal source/sink ratio (Tian *et al*., 1999). Deficiency of potassium occurs easily in Bt as compared to non Bt varieties because of more boll load in a shorter period and thus more nutrients including potash per unit time during boll formation (Wright, 1999). Growth and development of cotton is depressed by potassium deficiency affecting stems, leaves, roots and bolls; thus overall growth is stunted (Pettigrew and Meredith, 1997). Potassium deficiency affected cotton crop maturity due to early termination of reproductive growth and increased flowering rate in early season (Pettigrew, 2003). Potassium deficiency led to earlier cutout and caused fewer young bolls to survive (Reddy *et al*., 2000). Potassium deficiency reduced leaf phosynthesis, chlorophyll content, leaf area index, leaf and stem weights, plant height and stomatal conductance but enhanced mesophyll resistance synthesis of RuBP carboxylase and earliness of maturity (Hezhong *et al*., 2004).

Potassium deficiency at later stages can reduce cotton yield and quality (Gormus and Yucel, 2002) and resulted in premature senescence (Zhu *et al*., 2000). Recently cultivation of Bt cottons is increasing day by day due to more resistance against bollworms (Huang *et al*., 2003). It has been observed that at later stage during boll growth and development the supply of nutrients and saccharides may be shifted from leaves to the developing bolls; more sink and less source leads to the premature senescence of Bt cotton (Wright, 1999). New threat to cotton production is premature senescence due to increasing frequency and intensive cultivation system (Dong *et al*., 2005). In late season cotton's poor ability to take up potassium and other nutrients from soil might be possible reason of premature senescence (Brouder and Cassman, 1990), or unequal source/sink (Wright 1999). Premature senescence mostly occurs in Bt cotton cultivars than traditional varieties (Dong *et al*., 2005). After introduction of Bt cotton for commercial cultivation premature senescence has been occurring (Dong *et al*., 2006). Wright (1999) concluded that premature senescence occurred during boll filling period. It resulted in poor fibre properties and reduced lint yield (Wright, 1998). More bolls per plant and potassium deficiency caused premature senescence so, an understanding of leaf senescence may be helpful to avoid too early or too late senescence through appropriate management that would improve yield and

quality in cotton (Wright, 1999; Pettigrew, 2003). Too early senescence of a whole plant in cotton is referred to as premature senescence, which has been increased by commercial cultivation of Bt cotton (Dong *et al*., 2006).

Results of field and pot trials showed that more bolls, highest seed cotton yield and biomass were recorded with application of 225 kg K ha^{-1} (Zheng *et al*., 2013). More seed cotton yield (2418.55 kg ha^{-1}) was recorded with 125 kg K ha^{-1} followed by 2409.77 kg ha^{-1} with 62.5 kg K ha^{-1} and minimum (2192.98 kg ha^{-1}) was recorded in control i.e. with 0 kg K ha^{-1} (Rasool *et al*., 2010). More floral buds and bolls were obtained with application of potassium (Xia *et al*., 2011). Potassium application (47.4 kg ha^{-1}) significantly increased opened bolls per plant, seed index, lint percentage, average boll weight and lint index than the control treatment (Sawan *et al*., 2008). Combination of potassium and nitrogen application improved lint yield in less fertile field; however application of potassium improved lint yield in more fertile field (Dong *et al*., 2010).

Increase in leaf chlorophyll contents (15.63%) and more leaf protein contents (65 µg/g) were obtained with 200 mg K_2O kg^{-1} soil than control (Akhtar *et al*., 2009). Under potassium deficiency *Bacillus thuringiensis* (Bt) cotton cultivars senesce prematurely than conventional cultivars. Result of a hydroponic study showed that more dry matter and K+ was accumulated in Bt cotton seedlings with higher potash (high K+ 0.5 mM) than lower potash conditions (K+ 0.02 mM). Possible reason of premature symptoms in Bt cotton cultivars is its sensitively to potassium deficiency (Zhang *et al*., 2007). Premature senescence has been occurring in Australian cotton crops. A detailed comparison was made on ten pairs of adjacent or near adjacent (within 20 cm) plants, with and without symptoms. Plants with severe symptoms had 55–66% heavier total boll mass and their leaves had only about half the potassium (K) and three-quarters the phosphorus (P) concentration of unaffected plants. Hence, affected plants had less leaf K and P to meet the demand of a bigger boll load (Wright, 1999).

According to Yanshu *et al*. (2013), K-efficient cotton genotypes not only accumulated more potassium but showed higher nitrogen and phosphorus use efficiencies as well. According to Yang *et al*. (2011) less K efficiency (dry matter production divided by K accumulation) and K utilization efficiency (dry matter production divided by K concentration) was recorded in Bt cotton which might be due to the presence of foreign genes (CpTI and Bt). Oosterhuis (1994)

reported that potassium has the ability to produce turgor pressure which makes the fiber to elongate more. If K is deficient during fiber formation, it will cause reduction in turgor pressure which ultimately causes shorter length of fiber at maturity. It was observed that application of K increased fiber micronaire by 1 % and fiber elongation by 3%. Application of K had no effect on other characteristics of fiber (Pettigrew *et al*., 2005). Application of 250 kg K ha^{-1} improved fiber strength, fineness, maturity ratio and length. Positive correlation was found among quality traits of fiber and K contents of leaf tissues at bloom stage. These results indicated that adequate potassium availability plays an important in role producing quality cotton (Pervez *et al*., 2004). Response of K fertilizer to infestation of cotton leaf curl virus disease (CLCV); it was found that when K was applied at rate of 250 kg ha^{-1}, disease incidence was reduced by 12 to 38% (Pervez *et al*., 2007).

Result of an experiment showed that leaf Cry1Ac protein expression (four days prior to crop defoliation) in late growing season was not affected by applied potassium. Insect pest observations indicated that Cry1Ac protein expression was sufficient to control Heliothine larvae in the experiment (Rochester, 2006). Commercial cotton production is currently limited due to potassium (K) deficiency in China. Findings of an experiment showed that introduced genes (Bt and CpTI) that encode for insecticidal protein and their introduction processes surely affected tolerance to low potassium in Bt cotton (Li *et al*., 2008).

3.1. Experimental conditions

The conditions where experiments were carried out are briefly described as follows.

3.1.1. Experimental site

The experiments were conducted at Agronomic Research Area, University of Agriculture, Faisalabad, Pakistan. Two field trials were carried out in 2011 and repeated in 2012. The experimental site lies at 31° latitude and 73° longitude while the elevation of land is about 184.2 m above sea level.

3.1.2. Mechanical analysis of soil

The texture of experimental soil was loam. Before starting the experiment, soil samples were collected with standard methods for analyzing physico-chemical characters. For soaking the soil sample (50 g) overnight, one percent sodium hexameta-phosphate solution and distilled water was used. Then this was dispersed with stirrer and transferred to cylinder. Silt and clay particles were determined by using Bouyoucos hydrometer and soil textural class was determined with the help of international textural triangle (Moodie *et al.,* 1959).

3.1.3. Chemical analysis of soil

i) Saturation

Small amount of saturated soil paste was transferred to a china dish. It was dried at 105°C to obtain constant weight. The saturation percentage was calculated using formula (Handbook 60, Method 27a).

$$\text{Saturation percentage} = \frac{\text{(weight of wet soil – weight of dry soil)}}{\text{(weight of oven dried soil)}} \times 100$$

ii) Soil pH

After saturating the soil sample (250 g) in distilled water for one hour, the soil paste with soil to water ratio 1:10 was allowed to stand and pH was measured with the help of pH meter using buffer of 4.0 and 9.2 pH as a standard (Handbook 60, Method 21a).

iii) Electrical conductivity of soil (EC)

By using vacuum pump, the clear text of above mentioned paste was obtained. The electrical conductivity of soil sample was determined with the help of Digital Jenway conductivity meter Model 4070 (Handbook 60, Method 3a and 4b).

iv) Soil total nitrogen

Soil total nitrogen was measured by following the Kjeldhal's apparatus, the material containing 1 g ground soil (sieved through <2 mm sieve), 25 ml of concentrated H_2SO_4 and five gram of digestion mixture (K_2SO_4:$FeSO_4$:$CuSO_4$:, 85:10:05) was digested. Then distillation was carried out by taking 10 ml aliquot from digested mixture with the help of micro Kjeldhal's apparatus. The NH_3 gas evolved was absorbed in a receiver containing mixed indicator (Bromocresol green and methyl red). After the completion of distillation, contents of receiver were titrated against 0.1 N H_2SO_4 (Jackson, 1962) and nitrogen was calculated by the given formula.

% N = (Acid used for titration-blank) × 0.0014 × dilution factor × 100/ml of aliquot taken

v) Available phosphorus

Soil sample (5 g) was extracted with $NaHCO_3$ (0.5 M) and adjusted to pH 8.5 and 5 ml clear filtrate was taken in 100 ml volumetric flask and then 5 ml ascorbic acid was added for developing color. Volume was made up to mark, reading was recorded on spectrophotometer using 880 nm wavelength with the help of standard curve (Watanabe and Olsen, 1965).

vi) Extractable potassium

The same solution was used for extractable K determination by the help of flame photometer after calibrating with K solution (Handbook 60, Method 58, p.132).

vii) Organic matter

The ground and sieved (<2 mm sieve) soil (1 g) was mixed with 10 ml (1 N) potassium dichromate solution and added 25 ml of concentrated H_2SO_4. Then 150 ml of distilled water and 20 ml of 0.5 N ferrous sulphate solution were added and was titrated with 0.1 N potassium permanganate solution to pink end point (Moodie *et al*., 1959).

3.1.4. Meteorological data

The meteorological data for growth period of crop (Fig. 3.1) were collected from the meteorological observatory Crop Physiological Department, University of Agriculture, Faisalabad, Pakistan. The meteorological station is situated about 500 m away from the experimental site.

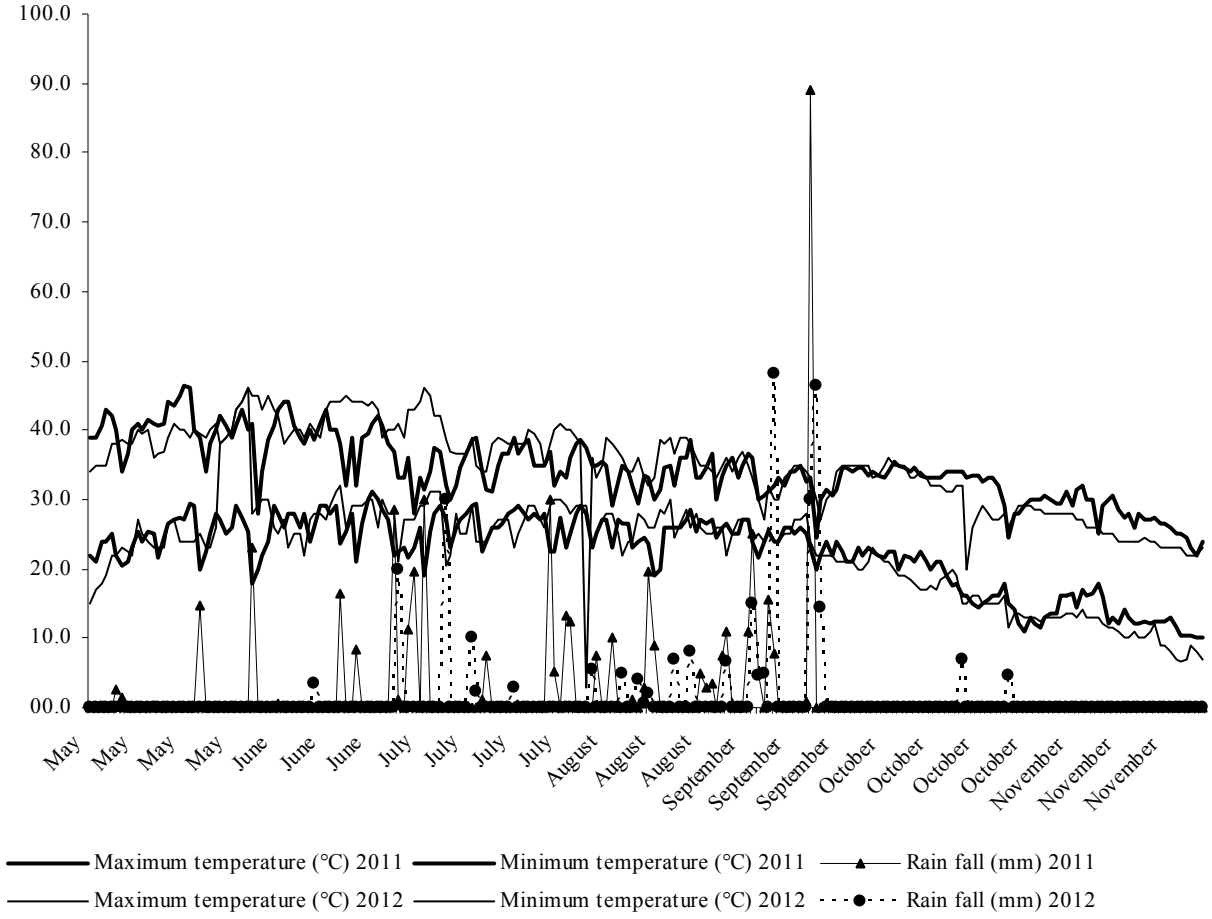


Fig-3.1: Weather conditions during cotton crop growth period

Table 3.1 Physico-chemical analysis of soil

		Experiment I				Experiment II			
Characteristics	**Unit**	**Value**				**Value**			
Study period		**2011**		**2012**		**2011**		**2012**	
Depth of sample	cm	1-15	15-30	1-15	15-30	1-15	15-30	1-15	15-30
Mechanical analysis									
Sand	%	50	48	50	49	48	49	50	50
Silt	%	22	23	21	22	21	22	23	21
Clay	%	28	29	29	29	31	29	27	29
Textural class		Loam				Loam			
Chemical analysis									
Saturation	%	32	34	38	35	36	33	37	34
EC	dS/m	2.02	1.79	1.90	1.76	1.86	2.10	1.41	1.47
pH	--	7.8	7.7	7.7	7.7	7.7	7.8	7.7	7.6
Organic matter	%	1.14	1.03	1.03	0.93	1.24	1.14	0.93	0.88
Total nitrogen	%	0.057	0.040	0.046	0.038	0.061	0.050	0.048	0.040
Available phosphorus	ppm	18.1	17.5	16.1	17.5	18.2	22.9	18.6	19.9
Available potassium	ppm	150	150	180	150	200	190	180	160

3.2. Experiments and treatments

The project comprised of the following two experiments. The detail of each experiment is as below.

3.2.1. Experiment I

Interactive effect of nitrogen rate and square/fruiting branch removal on Bt cotton

3.2.1.1. Treatments

Factor A: (Squares/fruiting branches removal)

F_1: No fruiting branch removal

F_2: Removal of first fruiting branch

F_3: Removal of first and second fruiting branch

F_4: Removal of all squares from first fruiting branch

F_5: Removal of all squares from first and second fruiting branch

Factor B: (Nitrogen levels)

N_1: 175 kg ha^{-1}

N_2: 225 kg ha^{-1}

N_3: 275 kg ha^{-1}

3.2.1.2. Crop husbandry

The experiment was planted on 15th May 2011 and on 16th May 2012 at Agronomic Research Area, University of Agriculture Faisalabad using randomized complete block design with factorial arrangement and replicated thrice. Seedbed was prepared by cultivating the field for two times with tractor-mounted cultivator each followed by planking. The crop was sown on loam soil. Sowing was done on well prepared ridges with the help of man power by maintaining 0.75 m row spacing and constant plant to plant distance of 0.30 m. Thinning was done at third true leaf stage. Whole of phosphorus @ 87 kg ha^{-1} (source SSP) and potassium @ 100 kg ha^{-1}

(source K_2SO_4) was applied at sowing and variable rates of nitrogen for different treatments were calculated based on the gross plot area and were applied in three equal splits viz. at sowing, after 35 days of sowing and after 65 days of sowing. Overall nine irrigations were applied and weeds were controlled by one pre-emergence herbicide at sowing and one post emergence herbicide (with help of protective shield) at 50 days after planting. Insecticides were applied as and when required. All other agronomic practices were kept normal and uniform for all the treatments. When seedlings were well established, five guarded representative plants were selected randomly in each plot and marked for identification.

3.2.2. Experiment II

Effect of early square/branch removal and rate of potash on phenology, yield and fiber qualities of Bt cotton

3.2.2.1. Treatments

Factor A: (Squares and fruiting branches removal)

F_1: No fruiting branch removal

F_2: Removal of first fruiting branch

F_3: Removal of first and second fruiting branch

F_4: Removal of all squares from first fruiting branch

F_5: Removal of all squares from first and second fruiting branch

Factor B: (Potassium levels)

K_1: 50 kg ha^{-1}

K_2: 100 kg ha^{-1}

K_3: 150 kg ha^{-1}

3.2.2.2. Crop husbandry

The crop was sown on 15th May 2011 and again on 16th May 2012 at Agronomic Research Area, University of Agriculture Faisalabad using randomized complete block design with factorial arrangement and replicated thrice. After seedbed preparation sowing was done on 0.75 m apart well prepared ridges while keeping plant to plant distance of 0.30 m. Thinning was done at third true leaf stage. Whole of phosphorus @ 87 kg ha^{-1} (source SSP) and variable rates of potassium (source K_2SO_4) for different treatments were calculated based on the gross plot area and nitrogen (in the form of urea) @ 175 kg ha^{-1} was applied in three equal splits viz. at sowing, after 35 days of sowing and after 65 days of sowing. Overall nine irrigations were applied and weeds were controlled by one pre-emergence herbicide at sowing and one post emergence herbicide (with help of protective shield) at 50 days after planting. Insecticides were applied as and when required. All other agronomic practices were kept normal and uniform for all the treatments. When seedlings were well established, five guarded representative plants were selected randomly in each plot and marked for identification.

3.2.3. Observations

Following parameters were recorded during the course of study for both the experiments.

A. Phenological related parameters

1) Number of days from planting to appearance of first floral bud

2) Number of days from planting to appearance of first flower

3) Number of days from planting to first boll splition

4) Boll maturation period (days)

B. Earliness traits (senescence indicators)

1) Node number for first fruiting branch

2) First fruiting branch height (cm)

3) Earliness index (%)

4) Seed weight (g)

5) Node above white flower

6) Node above cracked boll

C. Agronomic parameters

1) Average boll weight per plant (g)

2) Total number of bolls per plant

3) Number of opened bolls per plant

4) Number of unopened bolls per plant

5) Number of rotted bolls per plant

6) Number of insects' damaged bolls per plant

7) Number of plants attacked by CLCV

8) Number of monopodial branches per plant

9) Number of sympodial branches per plant

10) Seed cotton yield per plant (g)

11) Seed cotton yield (kg ha^{-1})

12) Plant height (cm) at appearance of first floral bud

13) Plant height (cm) at physiologically cut out stage

14) Plant height (cm) at last pick

D: Quality related parameters

1) Ginning out turn (%)

2) Fiber length (mm)

3) Fiber strength (g tex^{-1})

4) Fiber fineness (micronaire)

5) Fiber uniformity (%)

6) Fiber elongation (%)

7) Seed protein content (%)

8) Seed oil content (%)

E: Biochemical traits

1) Nitrogen concentration (%) in cotton leaf

2) Potassium concentration (mg g^{-1}) in cotton leaf

3) Cry1Ac protein concentration (µg g^{-1}) in pericarp of cotton boll

Procedure adopted for recording the parameters as follows.

A. Phenological related parameters

(1) Number of days from planting to appearance of first floral bud

Five guarded plants were selected at random from each plant. When first square of a size visible with naked eye appeared on 50 % of selected plants, number of days from planting were recorded. Average number of days taken to squaring was calculated.

(2) Number of days from planting to appearance of first flower

Number of days from planting to appearance of first flower was noted from the five guarded selected plants and average number of days taken to appearance of first flower was calculated.

(3) Number of days from planting to first boll splition

Number of days from planting to first boll splition was noted from the five selected plants and average number of days taken to boll splition was calculated.

(4) Boll maturation period (days)

Boll maturation period (days) was calculated by deducting number of days taken to flowering from number of days taken from planting to boll splition.

B. Earliness traits (senescence indicators)

(1) Node number for first fruiting branch

Number of the main stem node at which first fruiting branch arose was determined by designating node immediately above the cotyledonary scars as number two, and counting the successive ascending nodes until the one that gave rise to the first fruiting branch was reached.

(2) First fruiting branch height (cm)

Height of first fruiting branch (cm) was measured from pseudonode of five selected plants and finally average height of first fruiting branch was calculated.

(3) Earliness index (%)

It was measured with the help of following formula. This index is referred as maturity coefficient.

$$\text{Earliness index (\%)} = \frac{\text{Weight of seed cotton from first pick}}{\text{Total seed cotton weight from all picks}} \times 100$$

(4) Seed Index (100-seed weight g)

Weight of 100 seeds in grams is expressed as seed index. Thus to note seed index three samples of 100 seeds from each plot were weighed and finally averaged.

(5) Node above white flower

Weekly node above white flower measurements were initiated from five selected plants with the appearance of first flower (up to 100%) and continued until physiological cutout stage (NAWF=4) came, then average node above white flower was calculated.

(6) Node above crack boll

At twenty days interval node above crack boll measurements were initiated from five selected plants with the appearance of first boll splition (100% opening on guarded plants) and continued until (NACB=4) came, then average node above crack boll was calculated.

B. Agronomic parameters

(1) Average boll weight per plant (g)

Average boll weight (g) was calculated by dividing the total seed cotton yield per plant with respective number of opened bolls per plant.

(2) Total number of bolls per plant

Total number of bolls per plant was calculated by counting opened bolls, insects damaged bolls, rotten bolls and unopened bolls per plant of five randomly selected plants and average was calculated.

(3) Number of opened bolls per plant

By collecting the opened bolls per plant at first and second picking of five guarded plants.

(4) Number of unopened bolls per plant

Unopened bolls were counted on per plant basis of five selected plants after last picking and average was calculated.

(5) Number of rotted bolls per plant

Number of rotted bolls (on the basis of physical observation) of five selected plants was counted before first and second picking and after second picking then average was calculated.

(6) Number of insects' damaged bolls per plant

Number of insects' damaged bolls (on the basis of physical observation) of five selected plants was counted before first and second picking and after second picking (by cracking the bolls either it was insect damaged or healthy boll) then average was calculated.

(7) Number of plants attacked by CLCV

Number of plants attached by CLCV was counted from net plot and average was calculated on plot basis.

(8) Number of monopodial branches per plant

Monopodial branches of five randomly selected plants from each plot were counted and average number of monopodial branches for each plant was calculated.

(9) Number of sympodial branches per plant

Sympodial branches of five selected plants from each plot were counted and average number of sympodial branches per plant was calculated.

(10) Seed cotton yield per plant (g)

Seed cotton picked from five selected plants during all the pickings was weighed in grams using electric balance. Later the yield of seed cotton per plant was calculated.

(11) Seed cotton yield (kg ha^{-1})

Seed cotton yield (kg) per hectare was computed from seed cotton yield per plot.

(12) Plant height (cm) at appearance of first floral bud

Plant height (cm) of five randomly selected plants from each plot was measured at the appearance of first floral bud and average was calculated.

(13) Plant height (cm) at physiologically cut out stage

Plant height (cm) of five randomly selected plants from each plot was measured at physiological cutout stage and averaged.

(14) Plant height (cm) at last pick

Plant height (cm) of five randomly selected plants from each plot was measured after last pick.

C: Quality related parameters

(1) Ginning out turn (%)

(2) Fiber length (mm)

(3) Fiber strength (g/tex)

(4) Fiber fineness (Micronaire)

(5) Fiber uniformity (%)

(6) Fiber elongation (%)

Ginning out turn (%) was calculated after roller ginning approximately 100 g sample of the harvested seed cotton and GOT (%) was computed by using the following formula.

GOT (%) = (Weight of lint in sample / Weight of seed cotton in that sample) × 100

After ginning, 15 g lint samples were used for determination of above quality parameters. Lint quality parameters were determined in high volume instruments (HVI) at the laboratories of Fiber Technology Department in University of Agriculture, Faisalabad.

(7) Seed protein content (%)

Nitrogen content of cotton seed sample collected from each plot was determined by using micro-Kjeldhal methods (Bermmer, 1964) and then crude protein content was calculated by following formula.

Crude protein = Nitrogen × 6.25

(8) Seed oil content (%)

Cotton seed oil content (%) of each plot seed sample was measured by Soxhlet method described by Low (1990).

E: Biochemical traits

(1) Nitrogen concentration (%) in cotton leaf

At physiological cutout stage leaf sample were taken washed with distilled water, sun dried and then dried in oven at 70 °C till constant weight and ground with electrical grinder and sieved through <2mm sieve. The 0.5 g of sample was taken for nitrogen concentration determination following Kjeldahl method (Bremmer, 1964) detail is present in heading 3.1.3.

(2) Potassium concentration (mg g^{-1}) in cotton leaf

Potassium concentration (mg g^{-1}) in cotton leaf was determined by collection of subtended leaf from cotton plant at physiologically cutout stage. Leaf samples were washed with distilled water and sundried. After drying, ground the samples into powder form, weighed 0.5 g of this sample and digested it in diacid (HNO_3-$HCLO_4$) mixture in 2:1 ratio. Heated the samples at 60 °C for 15 min until reaction completed. Then increased heat to 120 °C and digested for 75 min or until sample cleared. Removed tubes from digester block, cooled and added distilled water to bring the solution up to 100 mL. Then standard was prepared from stock solution of 1000 ppm (AppliChem 1000 ppm) of 0, 25, 50, 100 ppm concentration and calibrated flame photometer (Sherwood Flame photometer 410) to make curve. Then multiplied the concentration of samples with dilution factor and divided by 1000 to convert ppm to mg g^{-1} (Gupta, 1999).

(3) Cry1Ac protein concentration in boll pericarp (µg/g)

Principle

Tissue extracts were added to wells coated with antibodies raised against Cry1Ac. Cry1Ac residues in the boll sample bind to the antibodies. A secondary antibody developed against Cry1Ac labeled with conjugate enzyme substrate was then added to the wells. After a wash step, an enzyme (alkaline phosphate) was added to develop the intensity of reaction which showed the color.

Sample preparation

At peak flowering stage ten flowers were tagged on five randomly selected guarded plants from each treatment taking as day one; after completion of 20 days samples of tagged bolls were collected in ice box in the morning. Samples were washed with distilled water for removing adherent material. From the pericarp of the boll 20 mg of fresh boll pericarp was taken with the help of punchers and put the punches in 1.5 ml eppendorf tube. Then ground with pestle

mortar and added 0.5 mL of 1x extraction/dilution buffer to the tube. Tissue was centrifuged at 13000 rpm using a motor driven pestle till it got extracted in to the buffer. Sample extracts were diluted at least at 1:11 prior to assay. For 1:11 dilution: added 0.5 mL 1x Extraction/Dilution buffer to dilution tubes labeled for each sample. Added 50 µL sample extract and mixed. To get a clear supernatant solution, tubes were spinned briefly in centrifuge. The supernatant solution is used to load the plate. Enzyme linked Immunosorbent Assay (ELISA) is the usual procedure followed for detection of Cry protein concentration in 20 days old cotton boll samples. The EnviroLogix (Cat # AP003) Cry1Ac plate is designed for quantitative laboratory estimation of Cry1Ac protein in cotton boll samples.

Preparation of Cry protein standard and samples

1. Added 100 µL of negative control, 100 µL of each calibrator and 100 µL of each sample extract to their respective wells. Follow this same order of addition for all reagents.
2. Thoroughly mixed the contents of the wells by moving the strip holder in a rapid circular motion on the bench top for a full 20-30 second. Care was taken when mixing to avoid cross-contamination.
3. Covered the wells with parafilm to prevent evaporation and incubated at ambient temperature for 15 minutes. Used an orbital plate shaker at 200 rpm.
4. Added 100 µL of Cry1Ab-Enzyme Conjugate to each well. Do not empty the well contents or wash the strips at this time.
5. Thoroughly mixed the contents of the wells as described in step 2. Care was taken during mixing to avoid cross-contamination.
6. Covered the wells with new parafilm to prevent evaporation and incubated at ambient temperature for one hour. Used an orbital plate shaker at 200 rpm.
7. After incubation, carefully removed the covering and vigorously shaked the contents of the wells into a sink or other suitable container. Flooded the wells completely with wash buffer, then shaked to empty. Repeated this wash step three times. Slaped the inverted plate several times on paper towel to remove as much water as possible.
8. Added 100 µL of substrate to each well.
9. Thoroughly mixed the contents of the wells, as in step 2. Cover the wells with new parafilm and incubated for 30 minutes at ambient temperature. Used an orbital plate shaker at 200 rpm.

10. Added 100 µL of stop solution (1.0 N Hydrochloric acid) to each well and mixed thoroughly. This turned the well contents yellow.
11. Read the plate within 30 minutes of the addition of stop solution at 450 nm.

3.2.4. Statistical analysis

Data collected on different parameters were analyzed statistically by using STATISTIX 10 program for analysis of variance and means were separated using Fisher's protected least significant difference (LSD) test at 5 % probability level (Steel *et al*., 1997). Figures were drawn by using Microsoft Excel programe.

3.2.5. Economic analysis

Economic analysis was performed on the basis of cost which varied in different treatments by following procedure given by CIMMYT (1988). Here, for each individual treatment, efforts were made to work out the contribution of gross income of crop. For this, cost of cotton production during 2011 and 2012 were calculated for factors which were kept uniform such as seedbed preparation, sowing, picking and land rent. Then expenditure for control (no fruiting branch removal) was calculated. Then variable cost incurring on different fruiting branches and/or square removal, nitrogen, phosphorus and potassium in each treatment was calculated separately. So, gross income was calculated on the basis of seed cotton yield per hectare according to present market value. The benefit cost ratio (BCR) for all individual treatments was calculated by the following formula.

$$BCR = \frac{\text{Gross income}}{\text{Total cost}}$$

The net income (Rs. ha^{-1}) was calculated by subtracting total variable cost (Rs. ha^{-1}) from gross income (Rs. ha^{-1}) for each treatment individually.

$$\text{Marginal rate of return (\%)} = \frac{\text{Change in net benefit}}{\text{Change in cost}} \times 100$$

4.1 Experiment I: Interactive effect of nitrogen rate and square/fruiting branch removal on Bt cotton

4.1.1. Phenological traits

Data in table 4.1 indicated that days to 50% squaring remained unaffected by fruiting branch and/or square removal (F), nitrogen rate (N) and their interaction however, year means differed significantly. Days to flower initiation, first boll splition and boll maturation period were significantly affected by fruiting branch and/or square removal (F) and nitrogen rates (N) while their interaction (F x N) was non-significant; however years showed non-significant effect for all these three parameters during both years of study. Comparison of treatments' means showed that days taken to open first flower (60.24 & 59.93) and first boll splition (93.71 & 93.37) were more in F_3 (removal of first and second fruiting branch) and less days to open first flower (48.62 & 49.06) and first boll splition (82.84 & 79.28) were recorded in F_1 (no fruiting branch removal), while years showed non-significant effect. Minimum boll maturation period (29.82 & 29.77 days) was recorded in F_5 (removal of all squares from first and second fruiting branch) as against maximum boll maturation period (34.22 & 34.71 days) in F_1 (no fruiting branch removal), which itself was not statistically different than F_2 (removal of first fruiting branch) and F_3 (removal of first and second fruiting branch) in case of boll maturation period (table-4.1). Among the nitrogen levels more number of days to first flower (58.04 & 57.44), days to first boll opening (91.70 & 90.73) and boll maturation period (33.66 & 33.56) were recorded in N_3 (275 kg ha^{-1}) followed by N_2 (225 kg ha^{-1}) and then in N_1 (175 kg ha^{-1}) during both study years (Table-4.1).

4.1.2. Discussion

Appearance of first flower can be altered by various factors like prevailing environmental condition (Shaheen *et al*., 2001), mineral nutrition (Saleem *et al*., 2010a) and cultivars (Anjum *et al*., 2001). When flower appears on cotton plant several hormonal changes occur leading to increased concentration of abscisic acid may be up to 100 folds (Dong *et al*., 2009). As abscisic acid has role in desiccation tolerance in seed, this higher concentration of abscisic acid in flower indirectly increases concentration of ethylene and form abscission zone on peduncle and flowers

Table 4.1: Effect of N level and removal of square/fruiting branch on phenological traits of cotton

	Days to squaring		Days to flowering		Days to boll splition		Boll maturation period (days)	
Square/branch removal (F)	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**
No fruiting branch removal (F_1)	38.88	37.40	48.62d	49.06d	82.84c	79.28d	34.22a	34.71a
Removal of first fruiting branch (F_2)	39.64	38.31	56.04b	55.82b	89.13ab	88.77ab	33.08ab	32.48ab
Removal of first and second fruiting branch (F_3)	39.48	37.88	60.24a	59.93a	93.71a	93.37a	33.46ab	33.80a
Removal of all squares from first fruiting branch (F_4)	39.13	37.82	52.22cd	52.95c	83.11c	82.80cd	30.88bc	30.13bc
Removal of all squares from first and second fruiting branch (F_5)	39.73	37.84	55.64bc	55.60bc	85.46bc	85.17bc	29.82c	29.77c
LSD (5%)	**NS**	**NS**	**3.730**	**2.655**	**4.904**	**5.019**	**2.954**	**2.600**
Nitrogen level (N)								
175 kg ha^{-1} (N_1)	37.81	37.78	50.92c	51.56c	81.65c	80.68c	30.73b	30.98b
225 kg ha^{-1} (N_2)	38.89	37.82	54.70b	55.02b	87.20b	86.24b	32.49ab	32.00ab
275 kg ha^{-1} (N_3)	41.42	37.94	58.04a	57.44a	91.70a	90.73a	33.66a	33.56a
LSD (5%)	**NS**	**NS**	**2.889**	**2.056**	**3.798**	**3.888**	**2.288**	**2.014**
Interaction (F × N)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Year mean	39.37a	37.85b	54.55	54.67	86.85	85.88	32.29	32.18
LSD (5%)	**1.399**		**NS**		**NS**		**NS**	

Means not sharing a letter in common differ significantly at 5% probability level.

NS= Non-significant,

start to drop. Manual removal of early squares increased the concentration of cytokinins and decreased concentration of abscisic acid in cotton and its effect remained effective till 45 days after the removal (Dong *et al*., 2009). In our study highest days to first flower were recorded with removal of first and second fruiting branch; and same was done with higher nitrogen dose. Similar trend was observed in days to first boll spilition. Removal of squares and/or floral buds as well as higher N dose not only delayed senescence but also increased node number for first fruiting branch and first fruiting branch height that may be due to increase in main stem node and increased internodal length.

4.1.3. Earliness traits

Data in table-4.2 depicted that fruiting branch/square removal (F) and nitrogen levels (N) have significant effects on node number for first fruiting branch and first fruiting branch height (cm) and earliness index; whereas seed index was significantly affected by nitrogen levels (N) and not by branch/square removal. Interactive response was non-significant, years also remained non-significant for all these four parameters. More nodes for first fruiting branch (9.66 & 9.77) and taller first fruiting branches (32.58 & 31.20 cm) were recorded in F_5 (removal of all squares from first and second fruiting branch) while F_4 (removal of all squares from fruiting branch) was statistically at-par with it and less nodes for first fruiting branch (8.26 & 8.06) and minimum first fruiting branch height (27.38 & 25.73 cm) was observed in F_1 (no fruiting branch removal). Minimum earliness index (47.32 & 47.40%) was recorded in F_5 (removal of all squares from first and second fruiting branch) as against maximum earliness index (52.73 & 52.85%) in F_1 (no fruiting branch removal). Among the nitrogen levels more node numbers for first fruiting branch (10.50 & 10.66), first fruiting branch height (35.30 & 34.01 cm), earliness index (50.11 & 50.94%) and seed index (7.84 & 7.89 g), were recorded with higher level of nitrogen application (275 kg N ha^{-1}) and less node numbers for first fruiting branch (7.65 & 7.49) first fruiting branch height (25.65 & 23.92 cm), earliness index (49.14 & 49.20%) and seed index (7.48 & 7.54 g) were recorded with lower level of nitrogen application (175 kg N ha^{-1}) as shown in table 4.2. Linear regression coefficient (R^2) of node number for first fruiting branch and first fruiting branch height (cm) with seed cotton yield per plant (g) was strong and positive as shown in figure-4.1.

Table 4.2: Effect of N level and removal of square/fruiting branch on earliness traits of cotton

	Node number for first fruiting branch		First fruiting branch height (cm)		Earliness index (%)		Seed index (g)	
Square/branch removal (F)	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**
No fruiting branch removal (F_1)	8.26c	8.06c	27.38c	25.73c	52.73a	52.85a	7.51	7.53
Removal of first fruiting branch (F_2)	8.44bc	8.40bc	28.36bc	26.82bc	50.20b	50.26b	7.61	7.69
Removal of first and second fruiting branch (F_3)	8.80bc	8.77bc	29.57bc	27.97bc	49.88b	48.37c	7.75	7.80
Removal of all squares from first fruiting branch (F_4)	9.15ab	9.15ab	30.65ab	29.20ab	48.05c	50.12b	7.53	7.59
Removal of all squares from first and second fruiting branch (F_5)	9.66a	9.77a	32.58a	31.20a	47.32c	47.40c	7.69	7.75
LSD (5%)	**0.859**	**0.991**	**2.911**	**3.154**	**1.638**	**1.665**	**NS**	**NS**
Nitrogen level (N)								
175 kg ha^{-1} (N_1)	7.65c	7.49c	25.65c	23.92c	49.14b	49.20b	7.48b	7.54b
225 kg ha^{-1} (N_2)	8.44b	8.34b	28.17b	26.62b	49.26b	49.27b	7.53b	7.58b
275 kg ha^{-1} (N_3)	10.50a	10.66a	35.30a	34.01a	50.11a	50.94a	7.84a	7.89a
LSD (5%)	**0.665**	**0.767**	**2.255**	**2.443**	**1.268**	**1.289**	**0.257**	**0.251**
Interaction (F × N)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Year mean	8.86	8.83	29.71	28.18	49.64	49.80	7.62	7.67
LSD (5%)	**NS**		**NS**		**NS**		**NS**	

Means not sharing a letter in common differ significantly at 5% probability level.

NS= Non-significant,

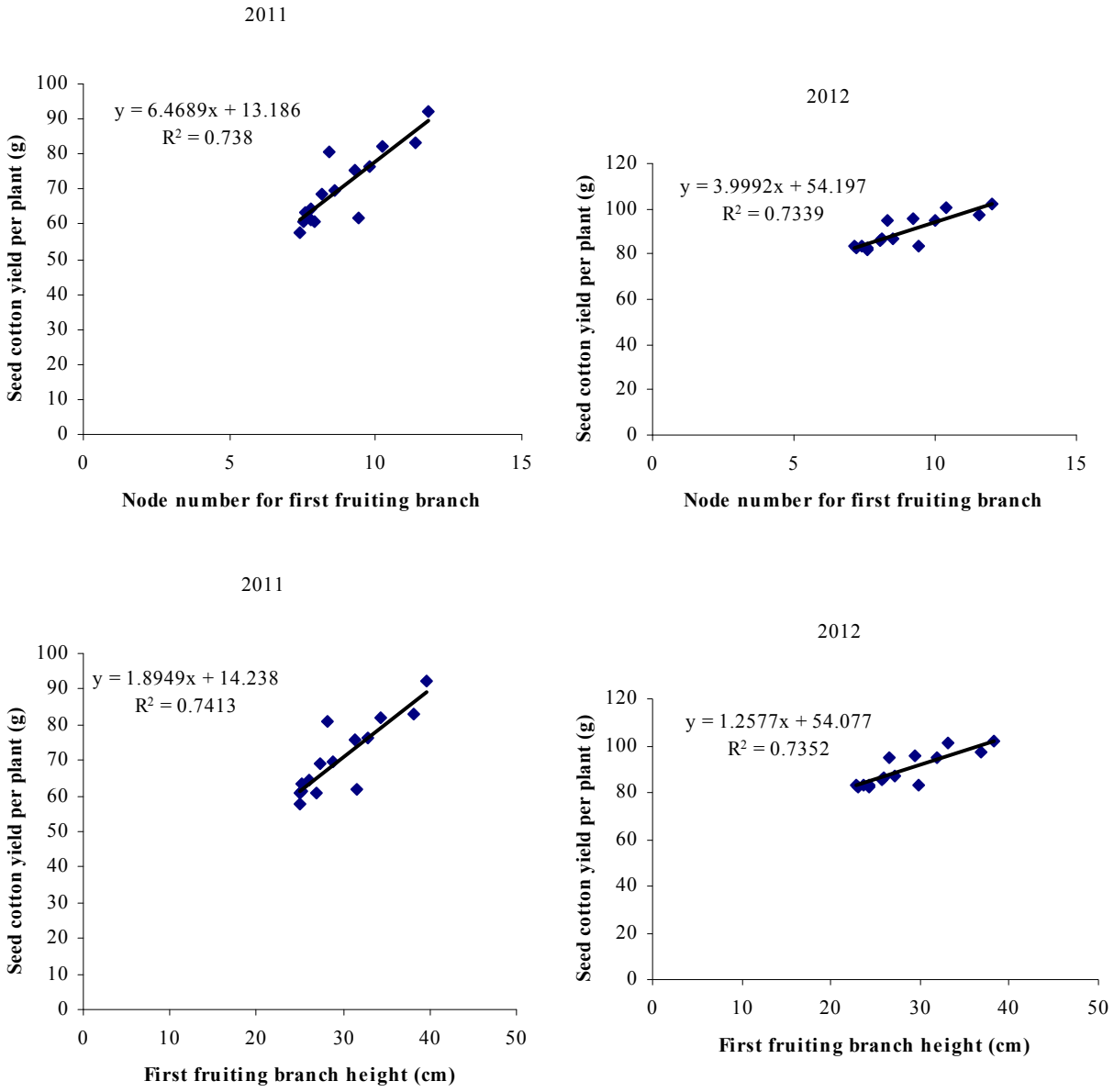


Fig. 4.1: Relationship of node number for first fruiting branch and first fruiting branch height (cm) with seed cotton yield per plant (g)

A gradual decrease towards the death of the cotton crop was measured in the form of node above white flower (NAWF). Taking observations on NAWF with weekly interval is a clear measurement of senescence in cotton crop. Periodic data pertaining to nodes above white flower of cotton as affected by fruiting branch/square removal and nitrogen rates during 2011 and 2012 are depicted in Fig. 4.2 & 4.3. There was visible difference in NAWF of the cotton crop with fruiting branch/square removal and nitrogen application. In the beginning, more NAWF was observed from 69 to 90 DAS (in 2011) and 68 to 82 DAS (in 2012) thereafter, it progressively decreased up to physiologically cut out stage (118 & 117 DAS) when node above white flower reached up to 4. Gradual decrease in NAWF during 2012 was recorded at 82 DAS but in 2011 gradual decrease in NAWF was recorded in 90 DAS. In fruiting branch/square removal more NAWF was observed with removal of all squares from first and second fruiting branch (F_5) followed by F_3 (removal of first and second fruiting branch), F_4 (removal of all squares from first fruiting branch), F_2 (removal of first fruiting branch) and F_1 (no fruiting branch removal) and among nitrogen application levels highest NAWF was observed with higher nitrogen dose (275 kg ha^{-1}) than medium (225 kg N ha^{-1}) and lower (175 kg N ha^{-1}) application.

Node above crack boll (NACB) was recorded three times during crop growth. Periodic data of NACB was affected by fruiting branch/square removal and nitrogen rates (fig. 4.4 & 4.5). The interactive response (F x N) was non-significant for NACB. More nodes above cracked bolls were recorded in F_5 (removal of all squares from first and second fruiting branch) and F_3 (removal of first and second fruiting branch) followed by F_2 (removal of first fruiting branch) and F_4 (removal of all squares from first fruiting branch) which were at-par with each other and minimum NACB (nodes above cracked bolls) were observed with no fruiting branch removal. Among nitrogen rates highest number of nodes above cracked bolls were recorded with the application of 275 kg N ha^{-1} followed by 225 kg N ha^{-1} and then by 175 kg N ha^{-1}. Periodic data showed that maximum NACB were observed at 132 & 124 DAS which gradually decreased reaching to a minimum of 5 NACB at 174 & 172 DAS during 2011 and 2012, respectively.

4.1.4. Discussion

Node number for first fruiting branch and first fruiting branch height are the morphological measures of earliness in cotton (Joham, 1979). Cotton cultivar matured earlier approximately 4 to 7 days by decrease in one node number of first fruiting branch (Ahmed and

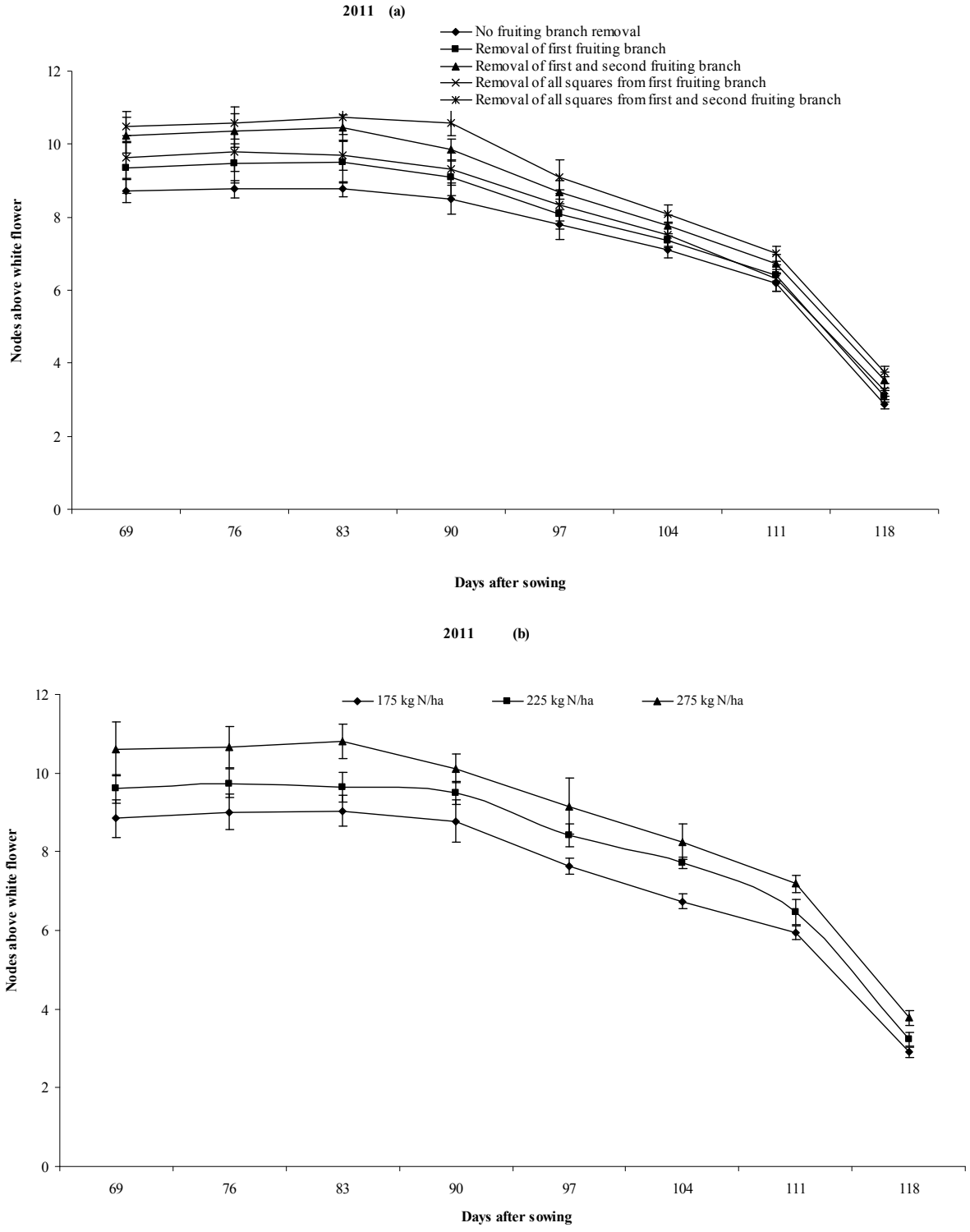
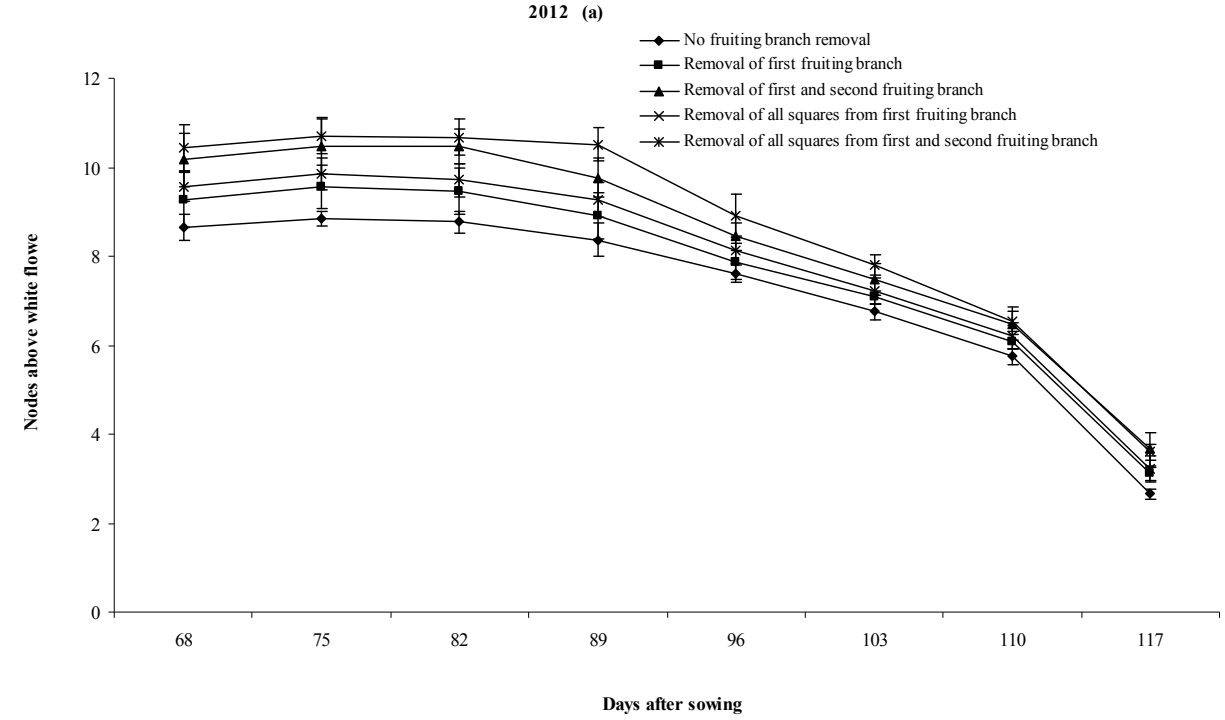


Fig. 4.2: Effect of (a) square/fruiting branch removal and (b) nitrogen levels on node above white flower in cotton during 2011



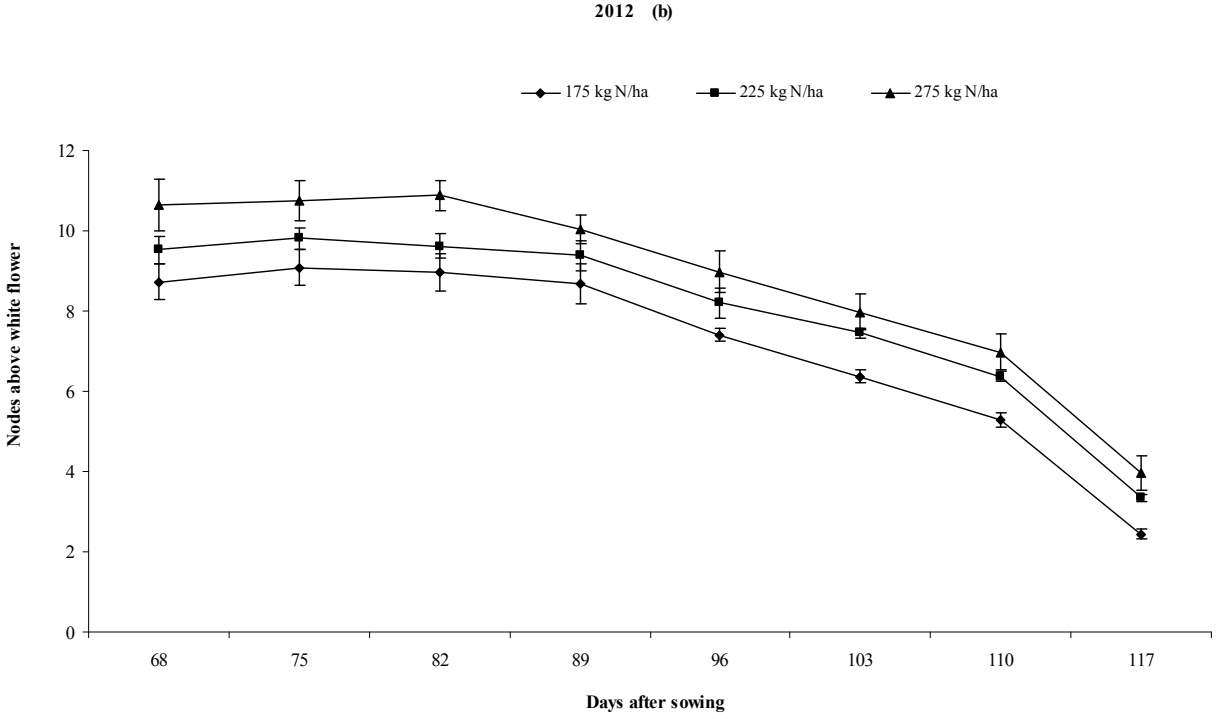


Fig. 4.3: Effect of (a) square/fruiting branch removal and (b) nitrogen levels on node above white flower in cotton during 2012

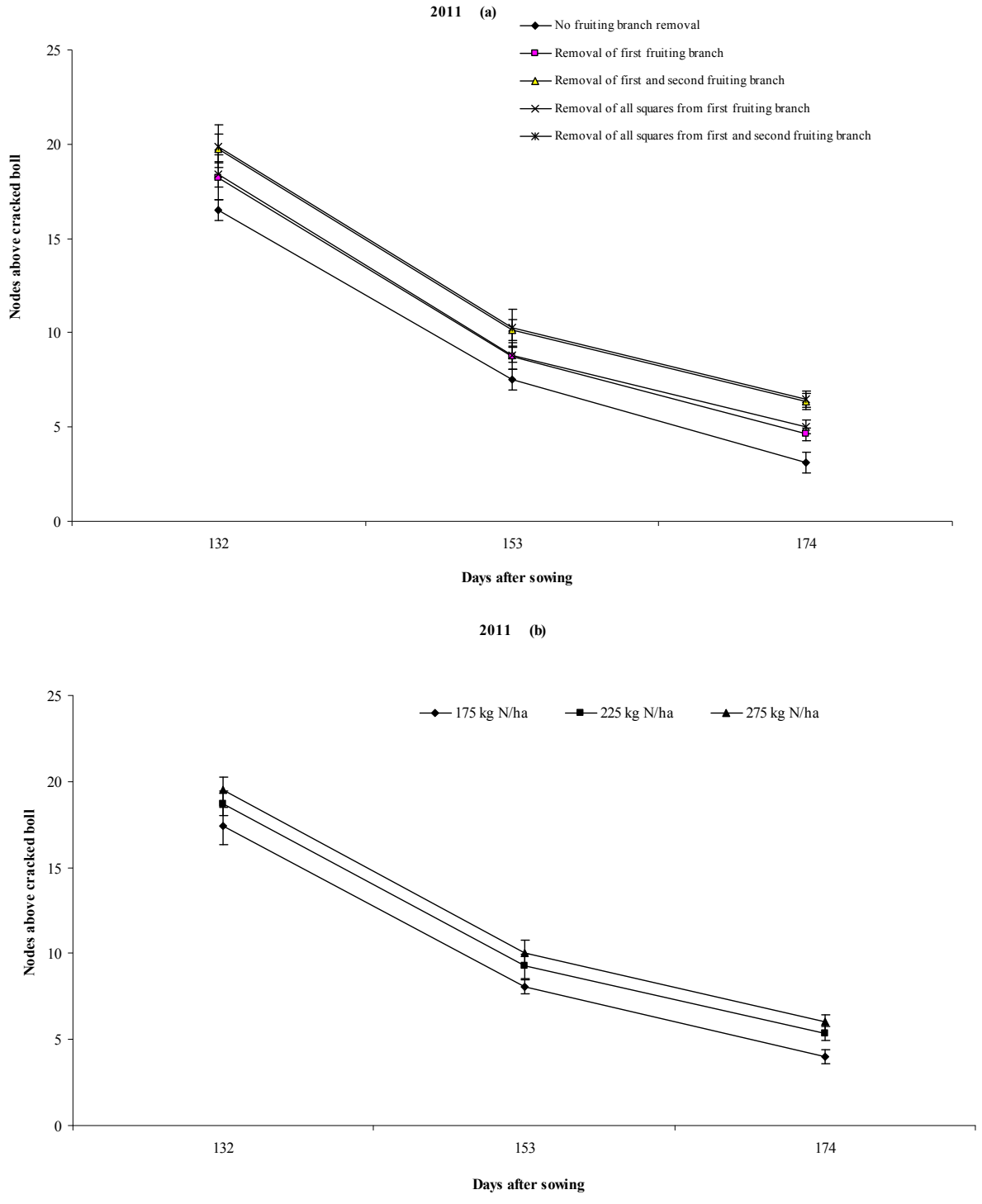


Fig. 4.4: Effect of (a) square/fruiting branch removal and (b) nitrogen levels on node above cracked boll in cotton during 2011

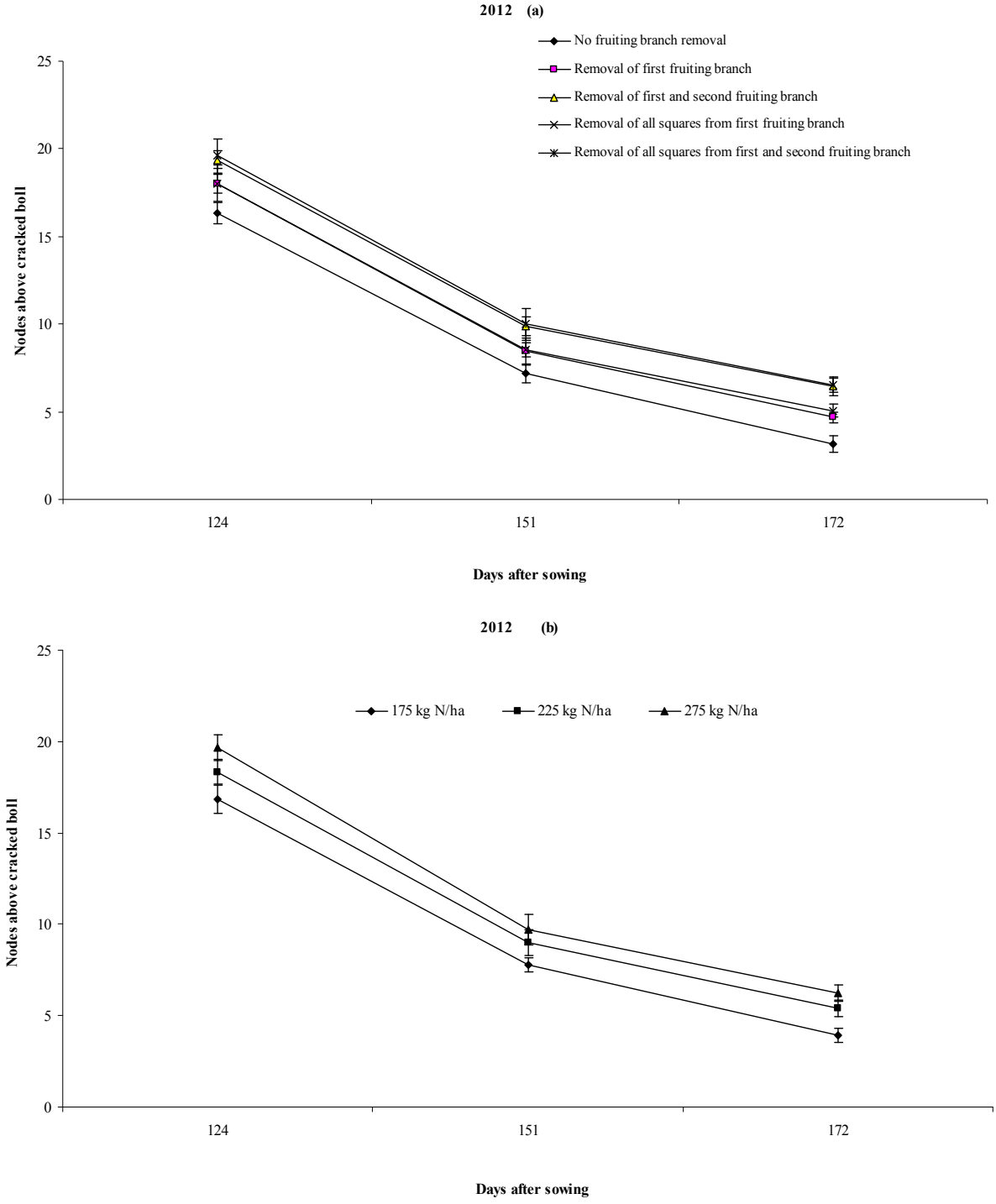


Fig. 4.5: Effect of (a) square/fruiting branch removal and (b) nitrogen levels on node above cracked boll in cotton during 2012

Malik, 1996). Less boll maturation period with removal of all squares from two early fruiting branches was due to more source availability at early stages which may help in rapid boll filling, but less earliness index in removal of all squares from first and second fruiting branch and with two early fruiting branch removal were due to more sink availability. Less boll maturation period with lower application of N caused reduced boll size, early boll filling with lower yield as compared to higher N rates (Saleem *et al*., 2010c). Premature senescence mostly occurring in commercially cultivated Bt cotton might be due to more sink and less source as a result of biological control of boll worm (Dong *et al*., 2006). In addition, senescence is usually associated with increase in ABA and ethylene and decrease in cytokinins (Buchanan, 1997; Oh *et al*., 1997). Among numerous factors such as nutrient deficiency (Wright, 1999), and alteration in phytohormones (Yong *et al*., 2000) especially cytokinins, ABA (Abscisic acid) and ethylene (Yang *et al*., 2004) caused initiation of senescence and its progress in cotton crop. Yield and quality of cotton was affected by both premature senescence and late maturity (Wright, 1999; Dong *et al*., 2006). For appropriate management in cotton it is very important to understand causes of senescence and it would help to overcome the losses due to premature and/or late senescence (Dong *et al*., 2009). Manual changes in plant architecture may enhance concentration of cytokinins and decrease concentration of ABA at early stage to enhance more vegetative growth at its initial stages of crop growth. Nodes above white flower were counted from peak flowering till physiological cutout stage to measure its senescence in field condition. More nodes above white flower were recorded with removal of all squares from first and second fruiting branch. The removal of early squares might have enhanced the concentration of growth promoting hormones whereas the subtended and main leaves of these branches also served as source of photosynthetic apparatus at initial stages, while minimum nodes above white flower in control treatment may be due to increased concentration of ABA at early stages when square was converted into young boll after fertilization. According to Dong *et al*. (2009) concentration of ABA is enhanced 100 fold in developing seed; because ABA has its role in desiccation tolerance hence it would prevent seed from desiccation injury that may be the cause of early senescence as it was observed in control treatment. At peak flowering stage nodes above white flower were more from mid July to first week of August and then gradually decreased so physiological cutout stage came at 2nd week of September in both years of study. Figure-1 showed temperature (maximum & minimum) was favorable for cotton growth from July to August during both years

of study. Senescence too early (premature senescence) or too late (late maturity) can be measured by nodes above white flower counts (Jones and Snipes, 1999). Guinn and his coworkers reported a series of detailed studies on the causes of cutout, hormonal effects and nutritional stress being the most important (Guinn, 1986). More nodes above cracked boll were observed in plots, where first and second fruiting branches were removed and supplied with higher dose of nitrogen, which is an indication of delay in senescence as compared with control. At squaring, differences in plant height were non-significant. Thereafter delay in senescence (increased plant height) was recorded in plots where either two early fruiting branches were removed or where all squares were removed from those two fruiting branches. The previous studies showed that fruit loss changes the partitioning of plant resources in support of vegetative growth (Sadras 1995; Jones *et al*., 1996). Preferred partitioning of photosynthates towards vegetative parts like root, stem, and leaf due to fruit losses might be responsible for the increased plant height (Sadras, 1996). Early fruit (sink) removal enhanced the vegetative growth and increased fruiting from later-developed positions also compensated earlier losses of fruit (Bednarz and Roberts 2001).

4.1.4. Agronomic traits

Year mean effect on average boll weight per plant was statistically non-significant but it was significant on number of unopened bolls per plant, number of rotted bolls per plant and number of insects' damaged bolls per plant with more values recorded in 2011 than during 2012. Fruiting branch and/or square removal (F) had non-significant effect, while nitrogen rates (N) has significant effect on average boll weight per plant, number of unopened bolls per plant and number of insects' damaged bolls per plant during both years of study. Interactive effect (F x N) was non significant on these parameters. While number of rotted bolls per plant remained unaffected with fruiting branch and/or square removal, nitrogen rates and their interaction (F x N). Comparison of treatments' means (table 4.3a) showed that more average boll weight per plant (3.26 & 3.33 g) was recorded in N_3 (275 kg N ha^{-1}) that was at-par with N_2 (225 kg N ha^{-1}) and less average boll weight per plant (2.92 & 2.99 g) was observed with low nitrogen rate (175 kg N ha^{-1}). Maximum number of unopened bolls per plant (9.05 & 6.90) was recorded with 175 kg N ha^{-1} that was at-par with medium dose (225 kg ha^{-1}) while less number of unopened bolls per plant (7.96 & 5.64) was recorded where N was applied at 275 kg N ha^{-1}. However maximum

Table 4.3a: Effect of N level and removal of square/fruiting branch on boll traits of cotton

	Average boll weight per plant (g)		Number of unopened bolls per plant		Number of rotted bolls per plant		Number of insects damaged bolls per plant	
Square/branch removal (F)	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**
No fruiting branch removal (F_1)	2.96	3.03	8.08	6.00	4.15	3.24	5.44	4.68
Removal of first fruiting branch (F_2)	3.08	3.14	8.33	6.13	4.22	3.40	5.55	4.80
Removal of first and second fruiting branch (F_3)	3.26	3.32	8.82	6.55	4.37	3.40	5.88	5.13
Removal of all squares from first fruiting branch (F_4)	3.08	3.14	8.44	6.26	4.28	3.37	5.66	4.91
Removal of all squares from first and second fruiting branch (F_5)	3.20	3.27	8.91	6.68	4.57	3.64	6.03	5.20
LSD (p=0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Nitrogen level (N)								
175 kg ha^{-1} (N_1)	2.92b	2.99b	9.05a	6.90a	4.16	3.22	5.38b	4.70b
225 kg ha^{-1} (N_2)	3.16ab	3.22ab	8.54ab	6.44a	4.24	3.40	5.69ab	4.70b
275 kg ha^{-1} (N_3)	3.26a	3.33a	7.96b	5.64b	4.57	3.61	6.08a	5.42a
LSD (p=0.05)	**0.243**	**0.241**	**0.601**	**0.530**	**NS**	**NS**	**0.487**	**0.520**
Interaction (F × N)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Year mean	3.11	3.18	8.52a	6.32b	4.32a	3.41b	5.71a	4.94b
LSD (5%)	**NS**		**0.315**		**0.229**		**0.298**	

Means not sharing a letter in common differ significantly at 5% probability level.

NS= Non-significant,

insects' damaged bolls per plant (6.08 & 5.42) were recorded with higher N dose and this damage decreased significantly with decreasing nitrogen (225 or 175 kg N ha^{-1}) (table 4.3a). Linear regression coefficient (R^2) for average boll weight per plant (g) vs. seed cotton yield per plant (g) was 0.69 & 0.64 during 2011 and 2012, respectively (Fig. 4.6).

Year mean effect on number of opened bolls per plant and total number of bolls per plant was significant with more number of opened bolls and less number of total bolls per plant in 2012 than 2011. Treatments' means as well as interaction (F x N) showed significant effect on number of opened bolls and total bolls per plant. Data given in table 4.3b show that maximum number of opened bolls per plant as well as total number of bolls per plant were recorded in plots where all squares were removed from first and second fruiting branches and cotton plants were supplied with higher N dose (275 kg N ha^{-1}) during both years of study. Application of F_3 (removal of first and second fruiting branch) and F_4 (removal of all squares from fruiting branch) treatments also performed equally well at higher N dose with respect to opened bolls and total bolls per plant. Differences among N rates were less marked in F_2 (removal of first fruiting branch) while in control (no fruiting branch removal) all the three nitrogen rates were statistically (P=0.05) same for the parameters under discussion. The trend was same during both study years. Linear regression coefficient (R^2) for opened bolls per plant vs. seed cotton yield per plant (g) was 0.73 & 0.99 for 2011 and 2012, respectively as shown in figure-4.6.

Year mean effect on number of plants attacked by CLCV per plot was non significant during 2011 and 2012. Nitrogen rate (N) has significant effect on number of plants attacked by CLCV per plot while fruiting branch and/or square removal (F) and their interaction (F x N) had non-significant effect (fig. 4.7). Figure showed that increase in nitrogen application increased number of plants attacked by CLCV per plot during both years of study.

Table 4.3b: Interactive effect of N levels and removal of square/fruiting branch on boll traits in cotton

	Opened bolls per plant		Total bolls per plant	
	2011	**2012**	**2011**	**2012**
No fruiting branch removal (F_1)				
175 kg ha^{-1} (N_1)	20.80e	23.26d	38.68de	37.56e
225 kg ha^{-1} (N_2)	21.00de	23.20d	38.69de	36.96e
275 kg ha^{-1} (N_3)	20.86de	23.60cd	38.47e	37.32e
Removal of first fruiting branch (F_2)				
175 kg ha^{-1} (N_1)	20.53e	23.60cd	39.04de	38.21de
225 kg ha^{-1} (N_2)	21.40de	24.20cd	38.98de	38.17de
275 kg ha^{-1} (N_3)	23.66bc	26.80b	42.06bc	41.10bc
Removal of first and second fruiting branch (F_3)				
175 kg ha^{-1} (N_1)	20.66e	23.33cd	39.46de	38.43de
225 kg ha^{-1} (N_2)	23.40c	26.86b	43.26ab	42.30abc
275 kg ha^{-1} (N_3)	25.13ab	28.46a	44.20ab	43.26ab
Removal of all squares from first fruiting branch (F_4)				
175 kg ha^{-1} (N_1)	21.20de	23.60cd	39.73de	38.23de
225 kg ha^{-1} (N_2)	22.53cd	24.60c	40.46cde	38.63de
275 kg ha^{-1} (N_3)	25.13ab	27.53ab	44.02ab	42.53ab
Removal of all squares from first and second fruiting branch (F_5)				
175 kg ha^{-1} (N_1)	21.66de	24.46cd	40.81cd	40.02cd
225 kg ha^{-1} (N_2)	23.73bc	27.00b	43.43ab	42.49ab
275 kg ha^{-1} (N_3)	25.46a	28.80a	44.98a	44.40a
LSD 5 %	**1.676**	**1.326**	**2.298**	**2.256**
Year mean	22.48b	25.28a	41.08a	39.97b
LSD 5 %	**0.378**		**0.602**	

Means not sharing a letter in common differ significantly at 5% probability level.

NS= Non-significant,

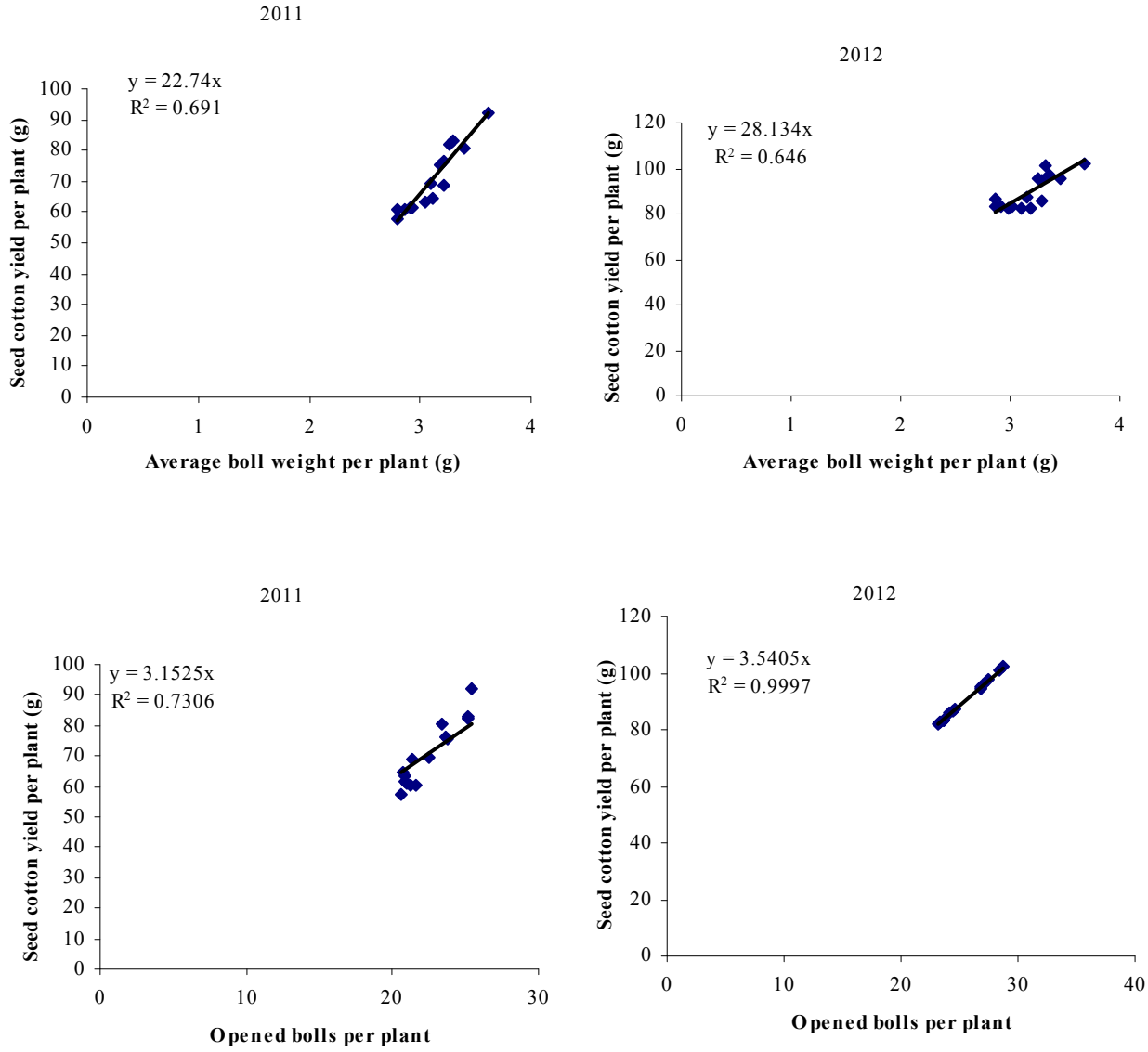


Fig. 4.6: Relationship of average boll weight per plant (g) and opened bolls per plant with seed cotton yield per plant (g)

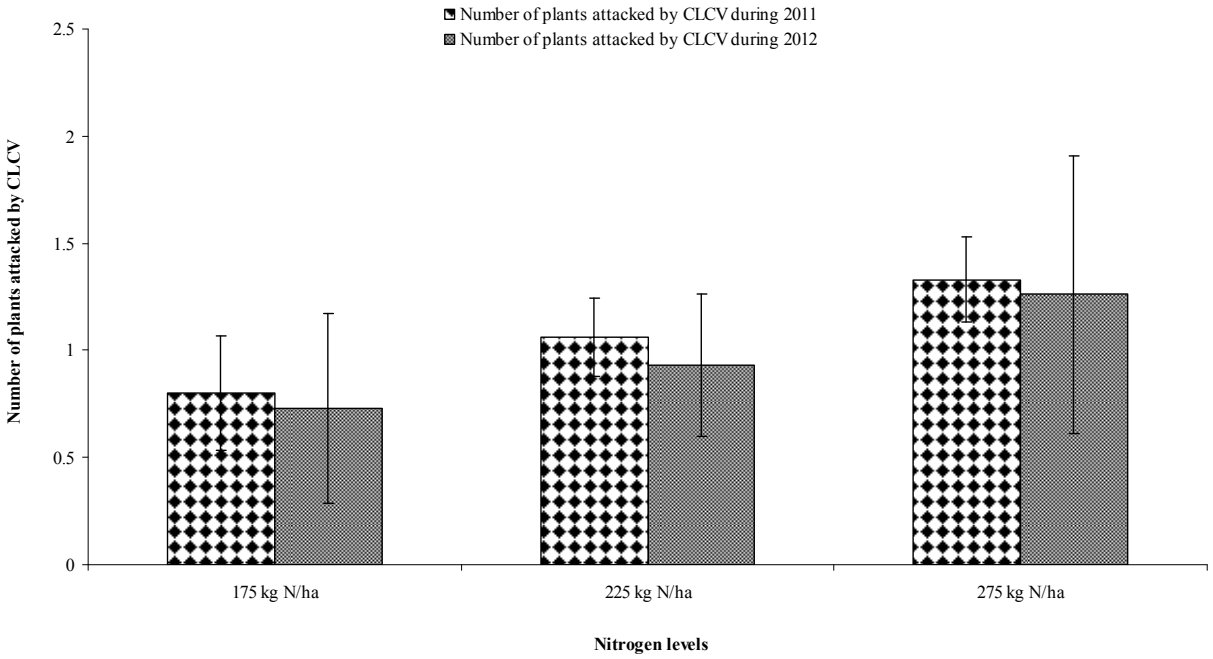


Fig. 4.7: Effect of nitrogen levels on number of plants attacked by CLCV in cotton.

Year mean effect on number of monopodial and sympodial branches per plant was significant showing more values in 2012 than 2011. Treatments' means and interaction showed significant effect on number of monopodial branches and number of sympodial branches per plant. Maximum number of monopodial branches per plant (2.86 & 3.33) was recorded with removal of all squares from first and second fruiting branch at higher nitrogen application (275 kg ha^{-1}) that was at par with medium dose (225 kg N ha^{-1}) of nitrogen application and less number of monopodail branches per plant (1.53 & 2.00) was recorded with low (175 kg N ha^{-1}) nitrogen application. Same trend was observed in F_3 (removal of first and second fruiting branch) and F_4 (removal of all squares from first fruiting branch) with higher, medium and low nitrogen rate; while in removal of first fruiting branch (F_2) higher dose of N produced significantly more branches than other two levels of nitrogen. In control (no fruiting branch removal) less number of monopodial branches pre plant (1.13 & 1.53) was recorded with lower nitrogen application (175 kg ha^{-1}) while medium and higher levels performed equal (table 4.4). For number of sympodial branches all treatments of square or branch removal performed best at higher nitrogen dose than at medium or lower levels however, in control (no square/branch removal) all three nitrogen levels were at par (P=0.05) with each other. Same trend was followed during both study years (table 4.4). Coefficient of determination (R^2) for monopodial and sympodial branches per plant vs. seed cotton yield per plant (g) was strong positive as shown in figure-4.8.

Year mean effect on seed cotton yield (per plant and per hectare) was significant. It was more in 2012 than 2011. Data pertaining to seed cotton yield as influenced by treatments' means as well as interaction (F x N) showed significant effect (table 4.5). Maximum seed cotton yield was recorded in plots where all squares were removed from first and second fruiting branches and cotton plants were supplied with higher N dose (275 kg N ha^{-1}) during both years of study. Application of F_3 (removal of first and second fruiting branch) and F_4 (removal of all squares from fruiting branch) treatments also performed equally well at higher N dose with respect to seed cotton yield. Trend was also similar in F_2 (removal of first fruiting branch) but its performance was poor than other treatments of branch or square removal. While in control (no fruiting branch removal) all the three nitrogen rates were statistically (P=0.05) same for the parameter under discussion. The trend was same during both study years. Coefficient of determination (R^2) of seed cotton yield per plant (g) with seed cotton yield (kg) per hectare was strong positive as shown in figure-4.9.

Table 4.4: Interactive effect of N levels and removal of square/fruiting branch on number of branches in cotton

	Monopodial branches per plant		Sympodial branches per plant	
	2011	2012	2011	2012
No fruiting branch removal (F_1)				
175 kg ha^{-1} (N_1)	1.13i	1.53h	17.86d	19.13d
225 kg ha^{-1} (N_2)	1.46ghi	1.86gh	18.06d	19.26d
275 kg ha^{-1} (N_3)	1.73efg	2.13efg	18.20d	19.46d
Removal of first fruiting branch (F_2)				
175 kg ha^{-1} (N_1)	1.33hi	1.80gh	17.60d	18.86d
225 kg ha^{-1} (N_2)	1.53fgh	2.00fg	18.20d	19.40d
275 kg ha^{-1} (N_3)	1.93de	2.40de	20.00c	21.26c
Removal of first and second fruiting branch (F_3)				
175 kg ha^{-1} (N_1)	1.86ef	2.33ef	17.46d	18.73d
225 kg ha^{-1} (N_2)	2.26cd	2.73cd	20.13c	21.40c
275 kg ha^{-1} (N_3)	2.40bc	2.86bc	21.40ab	22.66ab
Removal of all squares from first fruiting branch (F_4)				
175 kg ha^{-1} (N_1)	1.46ghi	1.93g	17.60d	18.86d
225 kg ha^{-1} (N_2)	1.93de	2.40de	18.40d	19.66d
275 kg ha^{-1} (N_3)	2.26cd	2.73cd	20.60abc	21.86abc
Removal of all squares from first and second fruiting branch (F_5)				
175 kg ha^{-1} (N_1)	1.53fgh	2.00fg	18.33d	19.53d
225 kg ha^{-1} (N_2)	2.73ab	3.20ab	20.20bc	21.46bc
275 kg ha^{-1} (N_3)	2.86a	3.33a	21.60a	22.86a
LSD 5 %	**0.384**	**0.378**	**1.253**	**1.238**
Year mean	1.89b	2.35a	19.04b	20.29a
LSD 5 %	**0.095**		**0.319**	

Means not sharing a letter in common differ significantly at 5% probability level.

NS= Non-significant,

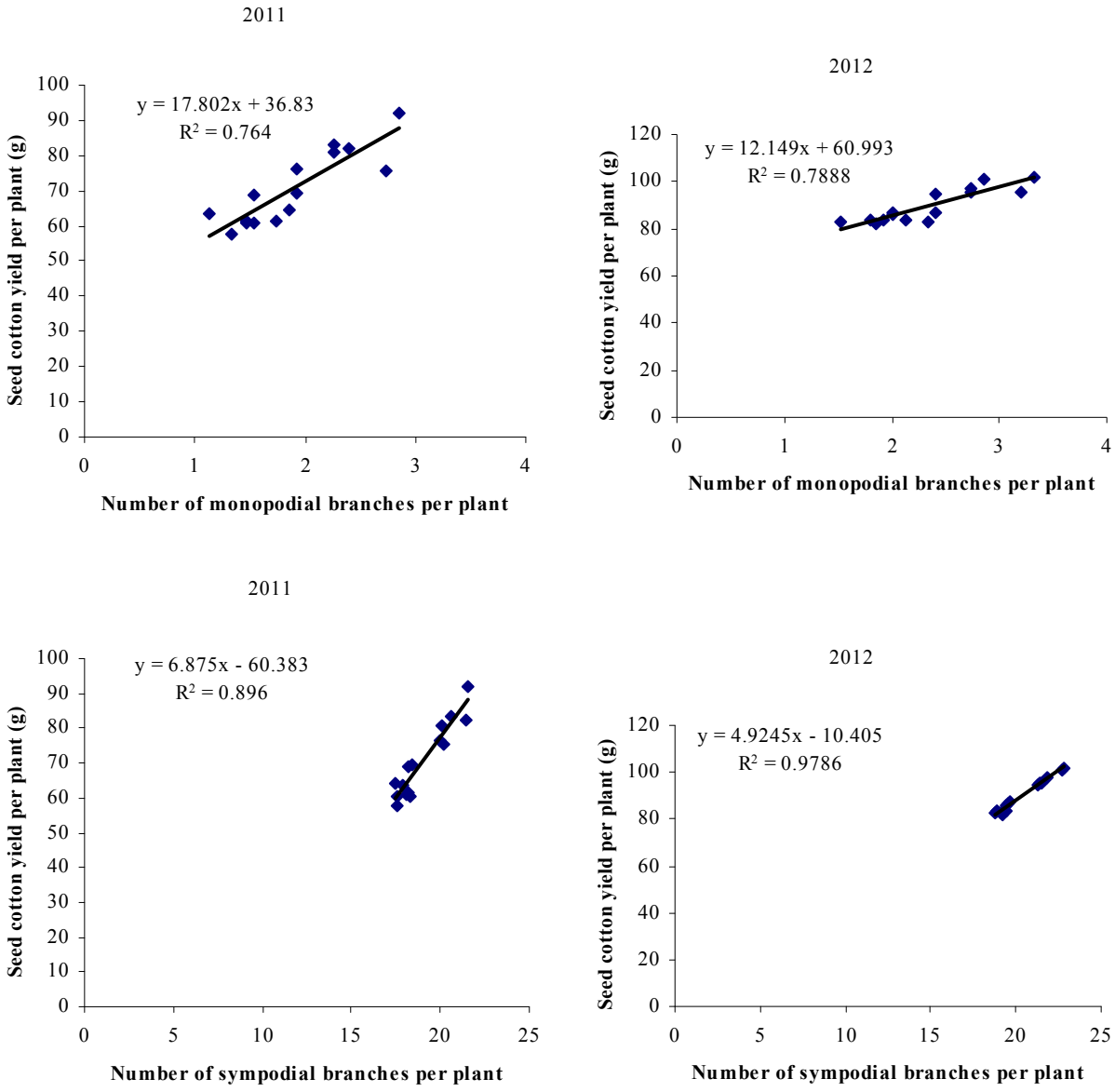


Fig. 4.8: Relationship of number of monopodial and sympodial branches per plant with seed cotton yield per plant (g)

Table 4.5: Interactive effect of N levels and removal of square/fruiting branch on seed cotton yield in cotton

	Seed cotton yield per plant (g)		Seed cotton yield (kg ha^{-1})	
	2011	**2012**	**2011**	**2012**
No fruiting branch removal (F_1)				
175 kg ha^{-1} (N_1)	63.45de	82.53cd	2621g	2996g
225 kg ha^{-1} (N_2)	61.22de	82.05d	2629g	3006g
275 kg ha^{-1} (N_3)	61.60de	83.49cd	2721fg	3107fg
Removal of first fruiting branch (F_2)				
175 kg ha^{-1} (N_1)	57.59e	83.45cd	2593g	2964g
225 kg ha^{-1} (N_2)	68.82cd	85.74cd	2890efg	3303efg
275 kg ha^{-1} (N_3)	76.44bc	94.61b	3229cde	3694cde
Removal of first and second fruiting branch (F_3)				
175 kg ha^{-1} (N_1)	64.42de	82.82cd	2862efg	3272efg
225 kg ha^{-1} (N_2)	80.72b	95.18b	3587abc	4100abc
275 kg ha^{-1} (N_3)	82.09ab	100.89a	3699ab	4257ab
Removal of all squares from first fruiting branch (F_4)				
175 kg ha^{-1} (N_1)	60.60de	83.41cd	2693fg	3080fg
225 kg ha^{-1} (N_2)	69.52cd	87.04c	3090def	3531def
275 kg ha^{-1} (N_3)	83.23ab	97.49ab	3648abc	4174ab
Removal of all squares from first and second fruiting branch (F_5)				
175 kg ha^{-1} (N_1)	60.66de	86.57cd	2696fg	3082fg
225 kg ha^{-1} (N_2)	75.52bc	95.55b	3371bcd	3850bcd
275 kg ha^{-1} (N_3)	92.15a	102.07a	3863a	4349a
LSD 5 %	**10.747**	**4.758**	**419.37**	**464.61**
Year mean	70.56b	89.52a	3079b	3518a
LSD 5 %	**2.157**		**110.09**	

Means not sharing a letter in common differ significantly at 5% probability level.

NS= Non-significant,

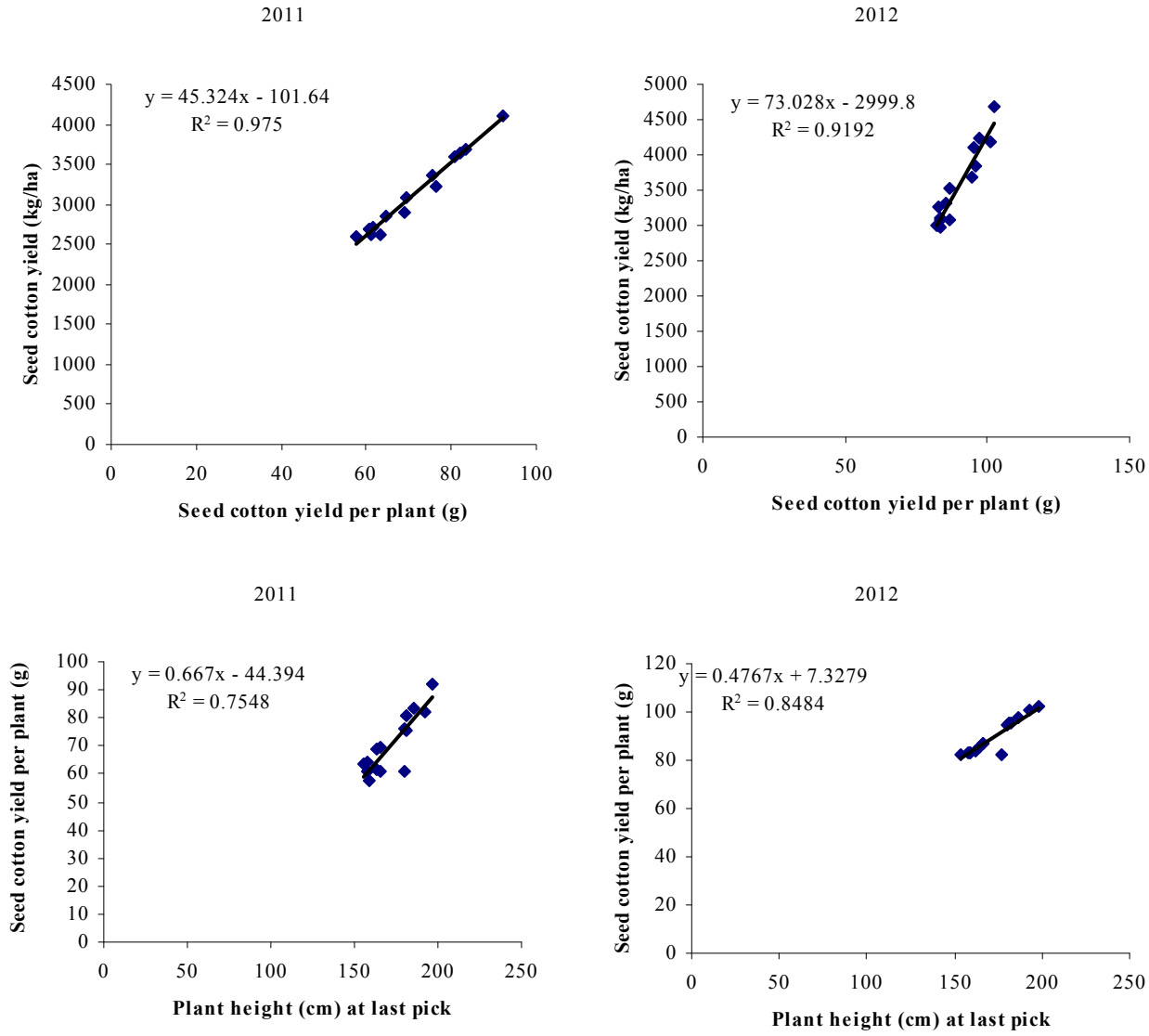


Fig. 4.9: Relationship between seed cotton yield per plant (g) vs. seed cotton yield per hectare (kg) and plant height (cm) last pick vs. seed cotton yield per plant (g)

Plant height remained non-significant at squaring stage (table 4.6) however, at physiological cutout stage and at last pick it was significantly affected by fruiting branch and/or square removal, nitrogen rates and their interaction as well during both years of study. Interactive effect (fig. 4.10) showed that cotton plants gained more height when we removed all squares from first and second fruiting branch (F_5) or when first and second fruiting branches were removed (F_3) at highest level of N application. Minimum plant height was observed with no fruiting branch removal closely followed by F_3 (removal of first and second fruiting branch) at lowest level of nitrogen application both at physiologically cutout stage and at last pick (maturity stage). Trend was almost similar during both years of study. There was strong positive relationship ($R^2 > 0.75$) between plant height (cm) at last pick with seed cotton yield per plant (g) as shown in fig. 4.9.

4.1.5. Discussion

Less boll maturation period with lower application of N caused reduced boll size and early boll filling with lower yield as compared to higher N rates (Saleem *et al*., 2010c). During peak squaring and boll development period total nitrogen contents in Bt cotton cultivars were higher than their parents; the uptake of nitrogen in the leaf of Bt cotton increased after introduction of the Bt gene (Chen *et al*., 2005b). Increase in boll weight and more opened bolls per plant were observed with increased nitrogen application from 95 to 143 kg ha^{-1} (Sawan *et al*., 2006). Square removal and leaf cut treatments were applied on Bt cotton; square removal increased boll size and reduced insecticidal protein content but leaf cut increased Bt toxin content and decreased boll size (Yonghui *et al*., 2009). Result of two years field trial on Bt cotton showed that two basal fruiting branches removal at squaring significantly increased lint yield (5.2 to 7.5 %), boll size (5.1 to 5.7 %), number of fruiting nodes, Cry1Ac protein in the fully expanded young leaves and Cry1Ac expression in terms of more insect pests resistance compared with their control treatment (Dong *et al*., 2008). Number of opened bolls and seed cotton yield per plant were increased by increasing nitrogen levels (Emara and Gammaal, 2012). In our study less number of opened bolls during 2011 may be due to more rainfall at early season which caused more young boll loss at initial stages that might be the possible reason of lower seed cotton yield per plant during 2011 than 2012 (fig. 3.1). Nitrogen application at 150 and 200

Table 4.6: Effect of N level and removal of square/fruiting branch on plant height (cm) at appearance of first floral bud

	2011	2012
Square/branch removal (F)		
No fruiting branch removal (F_1)	34.13	32.77
Removal of first fruiting branch (F_2)	34.75	34.08
Removal of first and second fruiting branch (F_3)	35.22	33.33
Removal of all squares from first fruiting branch (F_4)	35.04	33.53
Removal of all squares from first and second fruiting branch (F_5)	35.11	34.11
LSD (5%)	**NS**	**NS**
Nitrogen level (N)		
175 kg ha^{-1} (N_1)	34.14	32.93
225 kg ha^{-1} (N_2)	34.28	33.66
275 kg ha^{-1} (N_3)	36.13	34.10
LSD (5%)	**NS**	**NS**
Interaction (F × N)	**NS**	**NS**
Year mean	34.85	33.65
LSD (5%)		**NS**

Means not sharing a letter in common differ significantly at 5% probability level.

NS= Non-significant,

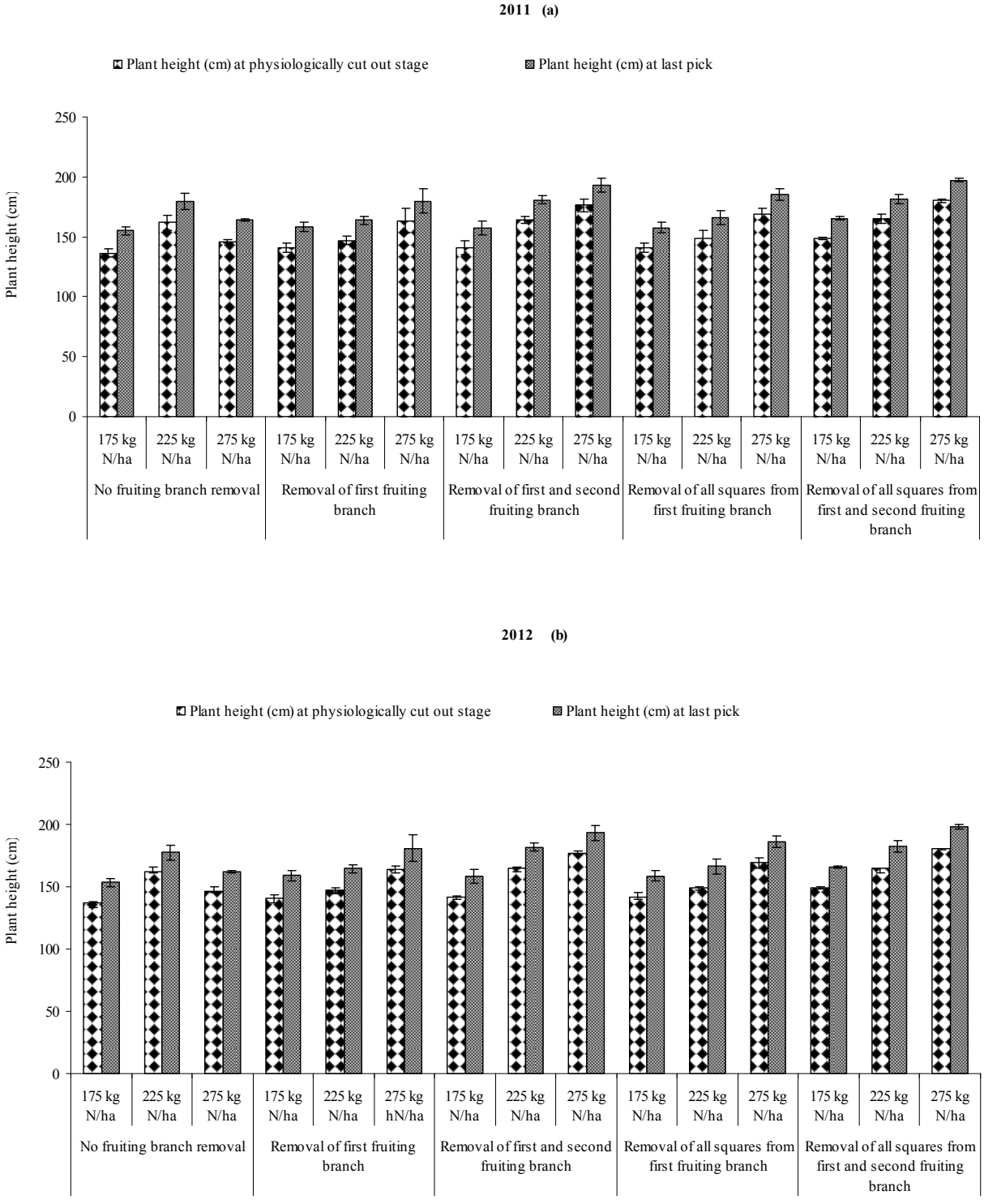


Fig. 4.10: Interactive effect of N levels and removal of squares and/or fruiting branches on plant height of cotton

kg ha^{-1} produced more bolls per plant, maximum number of seeds per boll, maximum average boll weight and seed cotton yield as compared to the lower rates (Khan and Dar, 2006).

Number of monopodial and sympodial branches are important yield determining traits in cotton. Increase in number of monopodial and sympodial branches with removal of squares from first and second fruiting branch or with removal of first and second fruiting branch was due to increase in main stem nodes by increasing plant height; manual removal of early squares increased the concentration of cytokinins and decreased concentration of abscisic acid in cotton and its effect remained effective till 45 days after the removal (Dong *et al*., 2009). Square and/or branch removal interacts with nitrogen and increased more vegetative growth at initial stages leading to more number of main stem nodes that may be responsible for more number of monopodial and sympodial branches per plant. Earlier findings showed that two basal fruiting branches removal at squaring increased plant height, leaf area and plant biomass as compared to control (Dong *et al*., 2008). We assumed that square and/or fruiting branches removal at early stage decreased sink/source ratio; less sink at early stage improved more vegetative growth (increased more source) that may be helpful at later stages for boll filling. Previous results showed that flower-bud removal can also increase single-leaf as well as canopy photosynthesis rate (Dumka *et al*., 2003); subtended leaf has 60 % role in boll filling that led to increased seed cotton yield per plant (g) and seed cotton yield per hectare in our study. Less seed cotton yield during 2011 may be due to more rain fall at initial stages of crop growth that led to shedding of more flowers at initial stages causing less bolls to contribute seed cotton yield (fig. 3.1). Increase in total number of bolls per plant may be due to more number of sympodial and monopodial branches per plant.

4.1.6. Quality traits

Year mean effect was significant on ginning out turn (%), fiber length (mm), fiber strength (g tex^{-1}), fiber uniformity (%), fiber elongation (%), seed protein content (%) and seed oil content (%) and non significant on fiber fineness (micronaire), with more values recorded in 2012 than 2011 except seed protein content (%) which showed opposite trend (table 4.7a and 4.7b).

Fruiting branch and/or square removal (F) showed non-significant effect on fiber strength, fineness, uniformity and elongation however, ginning out turn, fiber length, seed protein content and seed oil content were significantly affected by fruiting branch and/or square removal. Effect of nitrogen rates (N) was significant while interactive effect (F x N) was non-significant on all above mentioned quality traits (table 4.7a). Comparison of treatments' means (table 4.7a) showed more ginning out turn (GOT) in F_5 (removal of all squares from first and second fruiting branch) and F_3 (removal of first and second fruiting branch) being at par with each other but significantly better than F_1 (no fruiting branch removal), F_2 (removal of first fruiting branch) and F_4 (removal of all squares from first fruiting branch); the later three were also statistically similar.

Minimum fiber length was recorded in F_1 (no fruiting branch removal) while all other treatments of square/branch removal were equally good in this regard. Among nitrogen rates; these was observed an increasing trend in GOT and fiber length with increasing N rate (table 4.7a). Statistically maximum values for fiber length (26.10 & 27.36 mm) and fiber strength (23.62 & 24.74 g/tex) were exhibited by high N application (275 kg N ha^{-1}), whereas minimum fiber length (25.12 & 25.66 mm) and fiber strength (21.62 & 22.84 g/tex) were recorded with low N rate (175 kg N ha^{-1}). While maximum fiber fineness was obtained with lower N application and minimum fiber fineness was obtained with higher N application; trend was same during both years of study (table 4.7a). Linear regression coefficient (R^2) for ginning out turn (%) and fiber length (mm) vs. seed cotton yield per plant (g) was good and positive (figure 4.11).

Table 4.7(a): Effect of N level and removal of square/fruiting branch on quality traits of cotton

	Ginning out turn (%)		Fiber length (mm)		Fiber strength (g tex^{-1})		Fiber fineness (Micronaire)	
Square/branch removal (F)	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**
No fruiting branch removal (F_1)	37.17b	38.15b	24.67b	25.46b	22.2	23.38	5.34	5.36
Removal of first fruiting branch (F_2)	37.56b	38.81b	26.21a	26.96a	22.8	23.96	5.08	5.30
Removal of first and second fruiting branch (F_3)	39.43a	40.53a	25.51a	26.67a	22.6	23.91	5.45	5.52
Removal of all squares from first fruiting branch (F_4)	37.96b	39.08b	25.62a	26.56a	22.8	24.11	5.35	5.34
Removal of all squares from first and second fruiting branch (F_5)	39.54a	40.66a	25.78a	26.85a	22.5	23.70	5.48	5.37
LSD (5%)	**1.37**	**1.43**	**0.77**	**0.92**	**NS**	**NS**	**NS**	**NS**
Nitrogen level (N)								
175 kg ha^{-1} (N_1)	37.74b	38.71b	25.12b	25.66c	21.62c	22.84b	5.58a	5.60a
225 kg ha^{-1} (N_2)	38.16ab	39.10b	25.46b	26.49b	22.64b	23.86a	5.26ab	5.26b
275 kg ha^{-1} (N_3)	39.09a	40.53a	26.10a	27.36a	23.62a	24.74a	5.18b	5.28b
LSD (5%)	**1.06**	**1.11**	**0.59**	**0.72**	**0.89**	**0.89**	**0.323**	**0.274**
Interaction (F × N)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Year mean	38.33b	39.45a	25.56b	26.50a	22.63b	23.81a	5.34	5.41
LSD (5%)	**0.611**		**0.378**		**0.504**		**NS**	

Means not sharing a letter in common differ significantly at 5% probability level.

NS= Non-significant,

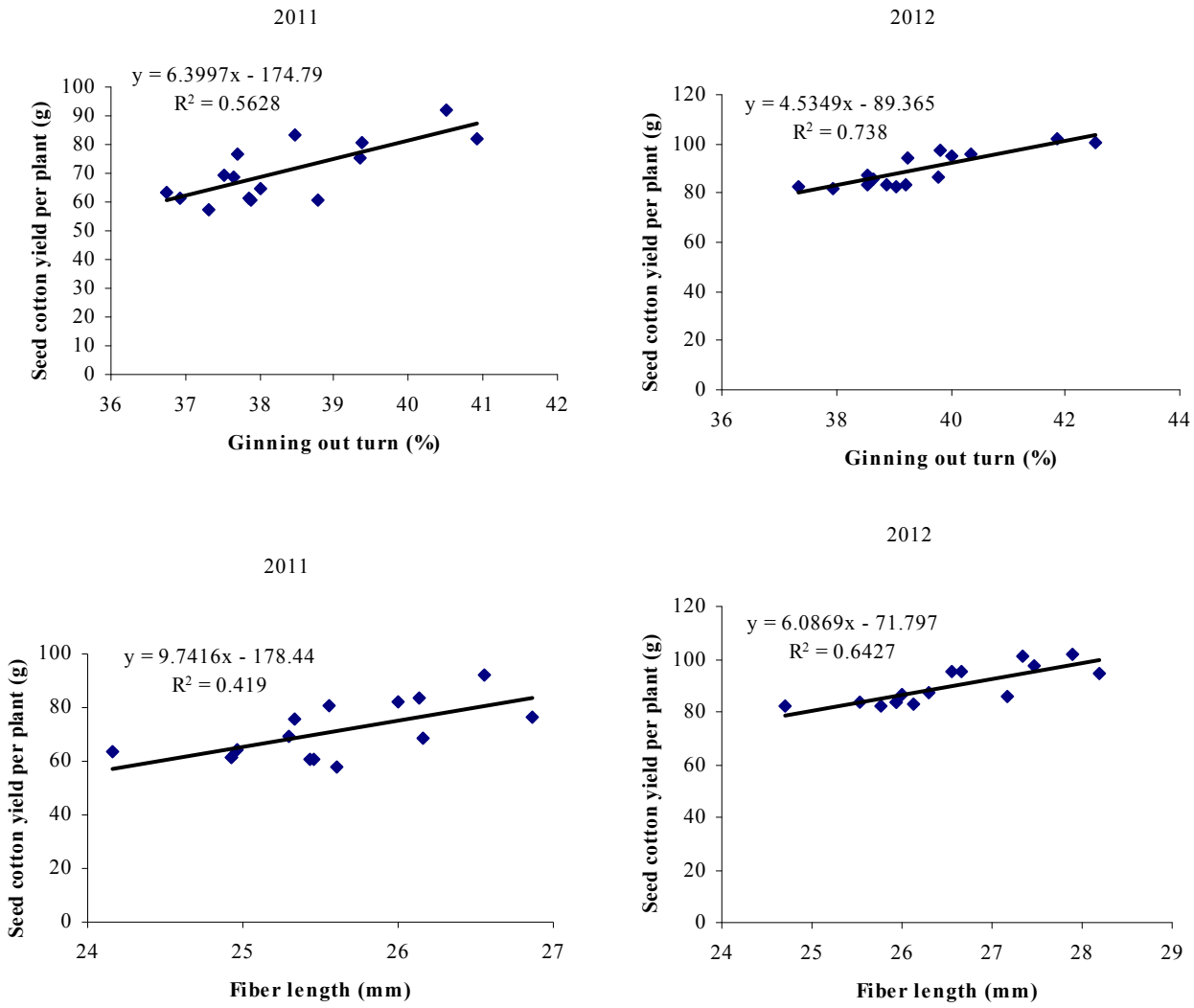


Fig. 4.11: Relationship of ginning out turn (%) and fiber length (mm) with seed cotton yield per plant (g)

The data given in table 4.7b show that more fiber uniformity was obtained with medium N application while higher and lower levels reduced fiber uniformity. Maximum fiber elongation (11.89 & 13.10 %) was obtained with N_3 (275 kg N ha^{-1}) that was at par with N_2 (225 kg N ha^{-1} and minimum fiber elongation (10.82 & 11.66 %) was recorded in N_1 (175 kg N ha^{-1}). Removal of all squares from first and second fruiting branch (F_5) and removal of first and second fruiting branch (F_3) increased seed protein content to a significant level than all other treatments during both years. Seed oil content was also higher in F_5 and F_3 but the differences were less marked from other treatments of square/branch removal. Among nitrogen levels increase in seed protein content and seed oil content was recorded with increasing N application from low to high (table 4.7b). A similar trend was observed during both years of study.

4.1.7. Discussion

Overall objective of cotton is lint production; increased lint yield indirectly enhanced ginning out turn (GOT). According to an estimate three percent increase in seed cotton yield could be expected with one percent increase in ginning out turn (Saleem *et al*., 2010b). Our experimental results also depicted positive relationship (R^2= 0.56 & 0.73) between ginning out turn and seed cotton yield per plant (g) as shown in figure 4.12. Effect of fruiting branch removal and/or square on fiber length was less markable as compared to no fruiting branch removal. While increase in nitrogen application increased fiber length; Bauer and Roof (2004) concluded that nitrogen application significantly improved fiber length compared to control. Yarn spinning ability depends on fiber strength; cotton with low strength or weak fiber is difficult to handle in manufacturing process. Usman *et al*. (2013) reported that fiber strength, length and fineness increased with application of nitrogen from 150 to 200 kg ha^{-1}. But in our study fiber fineness at medium and higher level of nitrogen application was at par with each other so, it can be concluded that fiber fineness did not significantly improve with increase in nitrogen application up to above certain limit. Same trend was observed in fiber uniformity and fiber elongation. Earlier findings showed that nitrogen rate had no effect on fiber uniformity (Hussain *et al*., 2000). Seed protein content increased with 50 kg ha^{-1} nitrogen application (Patil *et al*., 1997). Seed oil content was slightly decreased with an increase in the nitrogen level from 95.2 to 142.8 kg ha^{-1}, but seed oil yield had significantly increased (45.5 kg oil ha^{-1}), which is attributed to the significant increase in cotton seed yield (Sawan *et al*., 2007).

Table 4.7(b): Effect of N level and removal of square/fruiting branch on quality traits of cotton

	Fiber uniformity (%)		Fiber elongation (%)		Seed protein content (%)		Seed oil content (%)	
Square/branch removal (F)	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**
No fruiting branch removal (F_1)	48.9	50.12	12.0	12.56	13.12d	11.42c	15.27b	16.66c
Removal of first fruiting branch (F_2)	49.1	50.37	11.4	12.52	14.82cd	13.36b	17.11ab	18.50b
Removal of first and second fruiting branch (F_3)	46.8	48.04	11.2	12.73	17.01ab	15.06ab	18.72a	20.00a
Removal of all squares from first fruiting branch (F_4)	47.6	49.28	11.5	12.20	15.55bc	14.09b	17.05ab	18.44b
Removal of all squares from first and second fruiting branch (F_5)	49.2	50.64	11.1	12.67	17.49a	16.03a	18.72a	20.00a
LSD (5%)	**NS**	**NS**	**NS**	**NS**	**1.71**	**1.73**	**2.00**	**1.45**
Nitrogen level (N)								
175 kg ha^{-1} (N_1)	47.00b	48.08b	10.82b	11.66b	13.85c	12.39c	14.76c	16.13c
225 kg ha^{-1} (N_2)	50.10a	51.34a	11.78a	12.85a	15.60b	13.85b	17.43b	18.76b
275 kg ha^{-1} (N_3)	47.97b	49.65ab	11.89a	13.10a	17.35a	15.74a	19.93a	21.26a
LSD (5%)	**2.03**	**1.76**	**0.51**	**0.42**	**1.32**	**1.34**	**1.55**	**1.12**
Interaction (F × N)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Year mean	48.35b	49.69a	11.50b	12.54a	15.60a	13.99b	17.37b	18.72a
LSD (5%)	**1.067**		**0.263**		**0.764**		**0.769**	

Means not sharing a letter in common differ significantly at 5% probability level.

NS= Non-significant,

4.1.8. Biochemical traits

Data (table 4.8) pertaining to nitrogen concentration (%) in cotton leaf was significantly affected by fruiting branch and/or square removal and nitrogen doses while their interaction (F x N) was non-significant. Year effect was significant with more nitrogen content (%) during 2011 than 2012. Maximum nitrogen concentration (2.80 & 2.64 %) in cotton leaf was observed with removal of all squares from first and second fruiting branch closely followed by removal of first and second fruiting branch while minimum nitrogen concentration (2.10 & 1.94 %) in leaf was observed in control (no fruiting branch removal). Among nitrogen rates high nitrogen concentration (2.77 & 2.49 %) in cotton leaf was recorded with higher nitrogen application (275 kg N ha^{-1}), followed by medium N application(225 kg ha^{-1}) while low nitrogen concentration (2.21 & 2.07 %) in cotton leaf was recorded with the application of 175 kg N ha^{-1} (low N rate) during 2011 & 2012. Linear regression coefficient (R^2) for nitrogen concentration (%) in cotton leaf vs. seed cotton yield per plant (g) was strong positive as shown in figure-4.12.

Data in table 4.9 indicated that year mean effect was significant on potassium concentration (mg g^{-1}) with more values recorded in 2011 than during 2012. Interactive effect showed maximum increase in potassium concentration (mg g^{-1}) in cotton leaf with removal of all squares from first and second fruiting branch (F_5) and removal of first and second fruiting branch (F_4) with increased N application rate. It was followed by F_4 (removal of all squares from first fruiting branch), F_2 (removal of first fruiting branch) and then F_1 (no fruiting branch removal) where again more potassium concentration (mg g^{-1}) in cotton leaf was recorded with N_3 (275 kg N ha^{-1}) but it was at par with N_2 (225 kg N ha^{-1}) against the significantly less potassium concentration (mg g^{-1}) with N_1 (175 kg N ha^{-1}). Overall, control (no fruiting branch removal) remained at the bottom in accumulating potash in cotton leaf. Trend was same both years of study (table 4.9). Linear regression coefficient (R^2) for potassium concentration (mg g^{-1}) in cotton leaf with seed cotton yield per plant (g) was strong and positive as shown in figure-4.12.

Table 4.8: Effect of N level and removal of square/fruiting branch on N concentration (%) in cotton leaf

	2011	2012
Square/branch removal (F)		
No fruiting branch removal (F_1)	2.10d	1.94d
Removal of first fruiting branch (F_2)	2.37cd	2.17c
Removal of first and second fruiting branch (F_3)	2.68ab	2.41b
Removal of all squares from first fruiting branch (F_4)	2.48bc	2.25bc
Removal of all squares from first and second fruiting branch (F_5)	2.80a	2.64a
LSD (5%)	**0.284**	**0.232**
Nitrogen level (N)		
175 kg ha^{-1} (N_1)	2.21c	2.07c
225 kg ha^{-1} (N_2)	2.47b	2.28b
275 kg ha^{-1} (N_3)	2.77a	2.49a
LSD (5%)	**0.220**	**0.179**
Interaction (F × N)	**NS**	**NS**
Year mean	2.48a	2.28b
LSD (5%)		**0.055**

Means not sharing a letter in common differ significantly at 5% probability level.

NS= Non-significant,

Table 4.9: Interactive effect of N levels and removal of square/fruiting branch on K concentration (mg g^{-1}) in cotton leaf

	2011	2012
No fruiting branch removal (F_1)		
175 kg ha^{-1} (N_1)	5.00e	5.47d
225 kg ha^{-1} (N_2)	7.17c	7.70c
275 kg ha^{-1} (N_3)	7.41c	8.17c
Removal of first fruiting branch (F_2)		
175 kg ha^{-1} (N_1)	5.88de	7.17cd
225 kg ha^{-1} (N_2)	9.24b	10.42b
275 kg ha^{-1} (N_3)	9.59b	10.72b
Removal of first and second fruiting branch (F_3)		
175 kg ha^{-1} (N_1)	7.11cd	7.82c
225 kg ha^{-1} (N_2)	10.53b	12.07b
275 kg ha^{-1} (N_3)	12.60a	14.31a
Removal of all squares from first fruiting branch (F_4)		
175 kg ha^{-1} (N_1)	5.64e	6.82cd
225 kg ha^{-1} (N_2)	9.71b	11.24b
275 kg ha^{-1} (N_3)	10.24b	11.30b
Removal of all squares from first and second fruiting branch (F_5)		
175 kg ha^{-1} (N_1)	6.94cd	8.41c
225 kg ha^{-1} (N_2)	9.30b	11.01b
275 kg ha^{-1} (N_3)	12.66a	14.60a
LSD 5 %	**1.294**	**1.707**
Year mean	9.81a	8.60 b
LSD 5 %	**0.377**	

Means not sharing a letter in common differ significantly at 5% probability level.

NS= Non-significant,

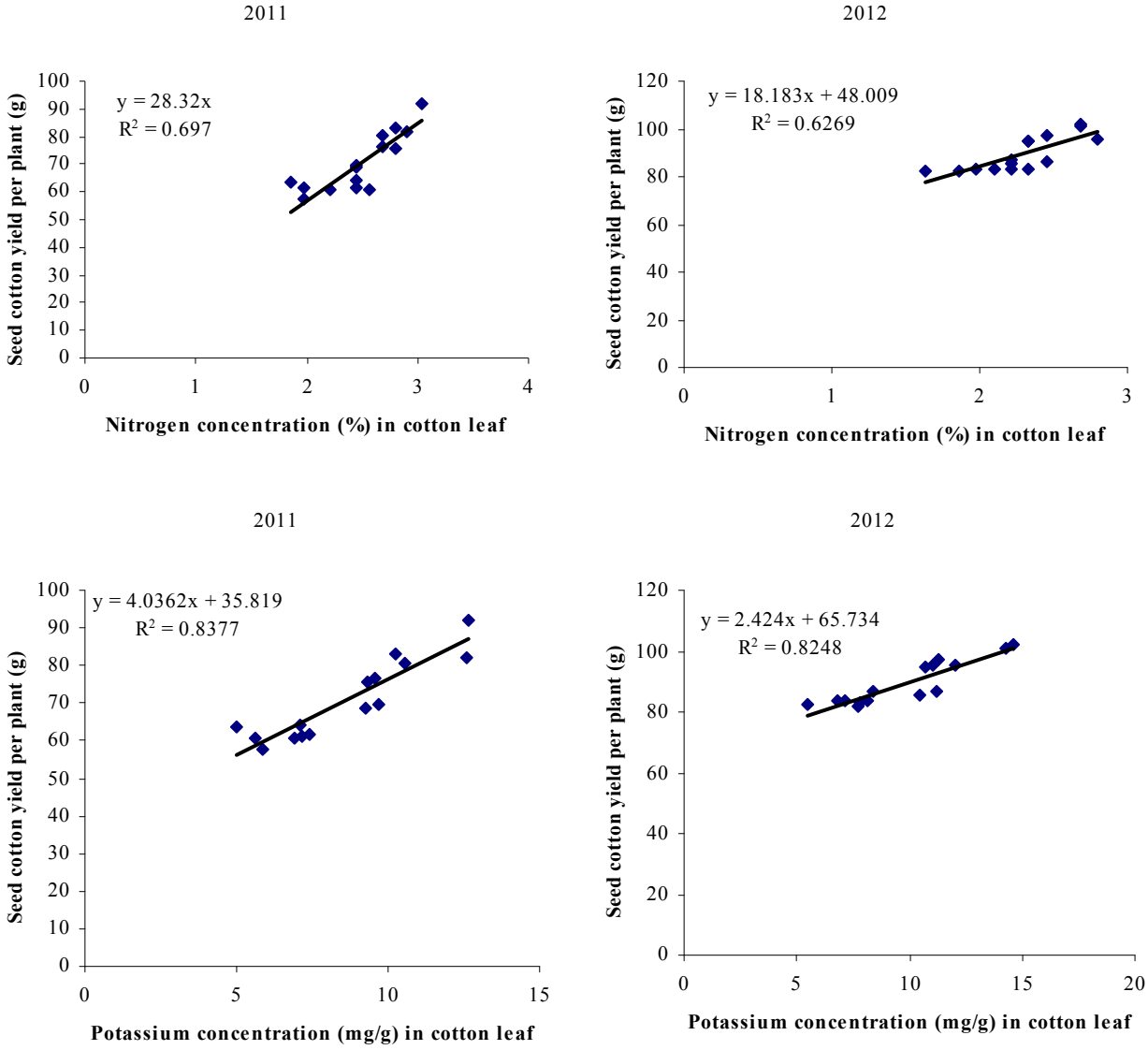


Fig. 4.12: Relationship of nitrogen concentration (%) and potassium concentration (mg/g) in cotton leaf with seed cotton yield per plant (g)

Year effect was significant on Cry1Ac concentration (µg/g) in pericarp of cotton boll; more Cry1Ac concentration (µg/g) in 20 days old boll pericarp of cotton was recorded during 2012 as compared to 2011 (table 4.10). During 2011 removal of all squares from first and second fruiting branch and removal of first and second fruiting branch when supplied with higher nitrogen dose (275 kg ha^{-1}) resulted in more Cry1Ac concentration, while less Cry1Ac concentration in 20 days old boll pericarp was recorded in control (no fruiting branch removal) with low nitrogen application (175 kg ha^{-1}). In 2012, trend was almost similar however, F_2 (removal of first fruiting branch) performed equally well as F_5 (removal of all squares from first and second fruiting branch) at higher N rate.

4.1.8. Discussion

Previous findings showed that two early basal fruiting branches removal had non-significant effect on K concentration (Dong *et al*., 2008) but their study was on two basal fruiting branches removal vs control; in our study increased K concentration in cotton leaf may be due to nitrogen application that enhanced K uptake in plant; According to Khalifa *et al*. (2012) highest K uptake efficiency of 42% was recorded when cotton crop was supplied with 240 kg N ha^{-1} x 0 kg K ha^{-1}. Another reason behind increased concentration of K in cotton leaf may be that when urea fertilizer is applied in soil it reduces soil pH where fixed potassium becomes exchangeable form and exchangeable potassium goes in solution form so cotton crop takes up more potassium. Cotton was planted with deficient (0 kg ha^{-1}N), adequate (100 kg ha^{-1}N) and excessive (200 kg ha^{-1}N) fertility. Leaves were collected from deficient, adequate, and excessive N fertility plots for Cry1Ac expression from nodes 8, 13 and 18. Leaf Cry1Ac protein expression was significantly increased with increasing N fertilizer application. As the season progressed, symptoms of N deficiency (light green plants) or excess N (dark green plants) were observed in the deficient and excessive N rate treatments. Moreover Cry1Ac protein expression was significantly higher in the older leaves (node 8) than the younger leaves (node 18) (Rochester, 2006). In this study there was more Cry1Ac concentration in treatment where two fruiting branches were removed or where all squares were removed from 1^{st} and 2^{nd} fruiting branches with higher nitrogen application; this may be due to more sources and less fruit at initial stage leading to good balance between source/sink at later stage.

Table 4.10: Interactive effect of N levels and removal of square/fruiting branch on Cry1Ac concentration (µg g^{-1}) in pericarp of cotton boll

	2011	2012
No fruiting branch removal (F_1)		
175 kg ha^{-1} (N_1)	0.51g	0.74f
225 kg ha^{-1} (N_2)	0.80efg	0.75f
275 kg ha^{-1} (N_3)	0.91def	1.61cd
Removal of first fruiting branch (F_2)		
175 kg ha^{-1} (N_1)	0.78efg	0.82f
225 kg ha^{-1} (N_2)	1.12cde	1.21e
275 kg ha^{-1} (N_3)	1.13cde	2.04ab
Removal of first and second fruiting branch (F_3)		
175 kg ha^{-1} (N_1)	0.88def	1.54cde
225 kg ha^{-1} (N_2)	1.31abc	1.60cd
275 kg ha^{-1} (N_3)	1.53ab	1.80bc
Removal of all squares from first fruiting branch (F_4)		
175 kg ha^{-1} (N_1)	0.64fg	1.19e
225 kg ha^{-1} (N_2)	1.18bcd	1.26de
275 kg ha^{-1} (N_3)	1.17cd	1.31de
Removal of all squares from first and second fruiting branch (F_5)		
175 kg ha^{-1} (N_1)	0.88def	1.53cde
225 kg ha^{-1} (N_2)	1.22abcd	1.83abc
275 kg ha^{-1} (N_3)	1.57a	2.16a
LSD 5 %	**0.355**	

Means not sharing a letter in common within a column differ significantly at 5% probability level.

NS= Non-significant,

4.1.9. Economic analysis

Net field benefit (NFB), benefit cost ratio (BCR) and marginal rate of return (MRR) were calculated on the basis of data of 2011 and 2012 (table 4.11 & 4.12). In 2011 Bt cotton grown with square removal from first and second fruiting branches at higher level of nitrogen application (275 kg ha^{-1}) gave maximum NFB of Rs. 168105 ha^{-1} followed by F_3 (removal of first and second fruiting branch) at higher N dose with NFB of Rs. 159156 ha^{-1}; while minimum NFB of Rs. 87353 ha^{-1} was associated with F_2 (removal of first fruiting branch) at low level of nitrogen application (table 4.11) almost similar trend was seen during 2012. Maximum BCR (2.65) was recorded with removal of all squares from first and second fruiting branch at higher level of nitrogen application followed by 2.60 (BCR) in removal of first and second fruiting branch at higher level of nitrogen application and minimum BCR (1.92) was recorded in control (no fruiting branch removal) at medium level of nitrogen application (225 kg ha^{-1}) during 2011 (table 4.11) while in 2012 maximum BCR (3.00) was recorded in F_3 (removal of first and second fruiting branch) at higher nitrogen dose (table 4.12). While maximum MRR (1839 & 2115) was recorded with removal of first and second fruiting branch with medium level (225 kg ha^{-1}) of nitrogen application, during both study years.

Table 4.11: Interactive effect of N levels and removal of square/fruiting branch in cotton on net income (Rs ha^{-1}), benefit cost ratio (BCR) and marginal rate of return (MRR%) during 2011

	Costs that vary	Marginal Costs	Total Cost	Gross income	Net income	Marginal net	BCR	MRR
No fruiting branch removal (F_1)								
175 kg ha^{-1} (N_1)	9130		92567	182945	90378		1.97	
225 kg ha^{-1} (N_2)	11739	2608	95176	183504	88327	-2050	1.92	-78
275 kg ha^{-1} (N_3)	14347	2608	97785	189925	92140	3812	1.94	146
Removal of first fruiting branch (F_2)								
175 kg ha^{-1} (N_1)	10201		93638	180991	87353		1.93	
225 kg ha^{-1} (N_2)	12809	2608	96247	201722	105474	18121	2.09	694
275 kg ha^{-1} (N_3)	15418	2608	98855	225384	126528	21053	2.27	807
Removal of first and second fruiting branch (F_3)								
175 kg ha^{-1} (N_1)	10379		93816	199767	105950		2.12	
225 kg ha^{-1} (N_2)	12988	2608	96425	250372	153947	47996	2.59	1839
275 kg ha^{-1} (N_3)	15596	2608	99034	258190	159156	5208	2.60	199
Removal of all squares from first fruiting branch (F_4)								
175 kg ha^{-1} (N_1)	11628		95065	187971	92905		1.97	
225 kg ha^{-1} (N_2)	14237	2608	97674	215682	118007	25101	2.20	962
275 kg ha^{-1} (N_3)	16845	2608	100283	254630	154347	36339	2.53	1393
Removal of all squares from first and second fruiting branch (F_5)								
175 kg ha^{-1} (N_1)	12877		96314	188180	91865		1.95	
225 kg ha^{-1} (N_2)	15486	2608	98923	235295	136372	44506	2.37	1706
275 kg ha^{-1} (N_3)	18094	2608	101532	269637	168105	31732	2.65	1216

Table 4.12: Interactive effect of N levels and removal of square/fruiting branch in cotton on net income (Rs ha^{-1}), benefit cost ratio (BCR) and marginal rate of return (MRR%) during 2012

	Costs that vary	Marginal Costs	Total Cost	Gross income	Net income	Marginal net	BCR	MRR
No fruiting branch removal (F_1)								
175 kg ha^{-1} (N_1)	9130		92567	209120	116553		2.25	
225 kg ha^{-1} (N_2)	11739	2608	95176	209818	114642	-1910	2.20	-73
275 kg ha^{-1} (N_3)	14347	2608	97785	216868	119083	4441	2.21	170
Removal of first fruiting branch (F_2)								
175 kg ha^{-1} (N_1)	10201		93638	206887	113248		2.20	
225 kg ha^{-1} (N_2)	12809	2608	96247	230549	134302	21053	2.39	807
275 kg ha^{-1} (N_3)	15418	2608	98855	257841	158985	24683	2.60	946
Removal of first and second fruiting branch (F_3)								
175 kg ha^{-1} (N_1)	10379		93816	228385	134568		2.43	
225 kg ha^{-1} (N_2)	12988	2608	96425	286180	189754	55185	2.96	2115
275 kg ha^{-1} (N_3)	15596	2608	99034	297138	198104	8349	3.00	320
Removal of all squares from first fruiting branch (F_4)								
175 kg ha^{-1} (N_1)	11628		95065	214984	119918		2.26	
225 kg ha^{-1} (N_2)	14237	2608	97674	246463	148789	28871	2.52	1106
275 kg ha^{-1} (N_3)	16845	2608	100283	291345	191061	42272	2.90	1620
Removal of all squares from first and second fruiting branch (F_5)								
175 kg ha^{-1} (N_1)	12877		96314	215123	118808		2.23	
225 kg ha^{-1} (N_2)	15486	2608	98923	268730	169806	50997	2.71	1954
275 kg ha^{-1} (N_3)	18094	2608	101532	303560	202027	32221	2.98	1235

4.2 Experiment II: Effect of early square/branch removal and rate of potash on phenology, yield and fiber qualities of Bt cotton

4.2.1. Phenological traits

Data in table 4.13 indicated that days to 50% squaring remained unaffected by fruiting branch and/or square removal (F), potassium rate (K), interaction (F x K) and by years also. Days to flower initiation, first boll spilition and boll maturation were significantly affected by fruiting branch and/or square removal (F) and potassium rates (K) while their interaction (F x K) was non-significant however, year means also differed significantly. Comparison of treatments' means showed that delayed flowering (63.77 & 61.55 days) and first boll spilition (98.51 & 95.11 days) were recorded in F_3 (removal of first and second fruiting branch) against the most early flowering (52.11 & 49. 75 days) and first boll spilition (85.11 & 81.80 days) in F_1 (no fruiting branch removal). Boll maturation duration was maximum in control (no fruiting branch removal) that was at par with F_2 (removal of first fruiting branch) and F_3 (removal of first and second fruiting branch) while minimum days for boll maturation were recorded with removal of all squares from first and second fruiting branch (F_5). Among potassium levels maximum days to flower and boll splition and minimum boll maturation period were recorded with higher level of potassium application while performance of medium and lower levels of potassium was statistically similar in this regard. Year mean effect on these above mentioned parameters was significant; 2011 recorded more days to flowering and boll splition while less boll maturation period than 2012.

4.2.2. Discussion

In our results days to squaring remained unaffected because there was no fruiting branch/ square removal at that time; after application of treatment (fruiting branch/square removal), days to flower initiation varied among different plots. Appearance of first flower can be altered by various factors like prevailing environmental condition (Sarwar *et al*., 2012), mineral nutrition (Saleem *et al*., 2010a) and cultivars (Anjum *et al*., 2001); in our experiment appearance of first flower was altered by changes in cotton plant architecture manually. Balance of growth promoter and growth inhibitor hormones is important for normal growth and development. When flower appears on cotton plant several hormonal changes occur leading to increased concentration of abscisic acid, may be up to

Table 4.13: Effect of K level and removal of square/fruiting branch on phenological traits of cotton

	Days to squaring		Days to flowering		Days to boll splition		Boll maturation period (days)	
Square/branch removal (F)	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**
No fruiting branch removal (F_1)	40.66	39.11	52.11c	49.75d	85.11d	81.80d	33.24a	34.57a
Removal of first fruiting branch (F_2)	41.37	39.82	59.48b	57.20b	93.82ab	90.51ab	31.86ab	33.44ab
Removal of first and second fruiting branch (F_3)	41.06	39.68	63.77a	61.55a	98.51a	95.11a	31.80ab	33.57ab
Removal of all squares from first fruiting branch (F_4)	41.95	39.22	55.75bc	53.40c	88.08cd	84.95cd	30.22bc	31.33bc
Removal of all squares from first and second fruiting branch (F_5)	41.31	39.97	59.06b	56.24bc	90.40bc	87.11bc	28.68c	30.20c
LSD (5%)	**NS**	**NS**	**3.759**	**3.639**	**4.849**	**5.103**	**2.928**	**2.924**
Potassium level (K)								
50 kg ha^{-1} (K_1)	40.16	38.02	55.48b	53.06b	87.58b	84.20b	32.74a	34.12a
100 kg ha^{-1} (K_2)	40.56	39.12	57.65b	55.24b	90.80b	87.66b	30.85ab	32.70ab
150 kg ha^{-1} (K_3)	43.10	41.54	60.98a	58.58a	95.17a	91.82a	29.89b	31.05b
LSD (5%)	**NS**	**NS**	**2.912**	**2.819**	**3.756**	**3.952**	**2.268**	**2.265**
Interaction (F × K)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Year mean	41.27a	39.56b	58.04a	55.63b	91.18a	87.89b	31.16b	32.62a
LSD (5%)	**NS**		**1.590**		**2.156**		**1.266**	

Means not sharing a letter in common differ significantly at 5% probability level.

NS= Non-significant,

100 folds (Dong *et al*., 2009). By manual removal of fruiting branch/square at early stages we might have indirectly decreased concentration of growth inhibiting hormones thus delaying its reproductive growth. As abscisic acid has role in desiccation tolerance in seed, this higher concentration of abscisic acid in flower indirectly increases concentration of ethylene and forms abscission zone on peduncle and flowers start to drop. Manual removal of early squares increased the concentration of cytokinins and decreased concentration of abscisic acid in cotton and its effect remained effective till 45 days after the removal (Dong *et al*., 2009). In our study highest days to first flower were recorded with removal of first and second fruiting branch; and same was done with higher potassium dose. Similar trend was observed in days to first boll spilition. Delayed squaring, flowering and boll splition in 2011 than 2012 might be due to more rainfall at initial stage (Fig. 3.1) while more boll maturation period during 2012 might be rainfall in later stage.

4.2.3. Earliness traits

A perusal of table 4.14 indicates more nodes for appearance of first fruiting branch (10.86 & 9.44) and more height for first fruiting branch (35.64 & 30.88 cm) in F_5 (removal of all squares from first and second fruiting branch) although it did not differ significantly (P=0.05) from F_4 (removal of all squares from fruiting branch). Control (no fruiting branch removal) recorded minimum values for these parameters and was similar with plants where we removed first fruiting branch. According to data presented in table 4.14 increasing potassium from 50 to 150 through 100 kg ha^{-1} delayed onset of first fruiting branch and also increased its height. Year mean effect was significant on node number for first fruiting branch and first fruiting branch height, with more values recorded during 2011 than 2012.

Table 4.14 depicted that fruiting branch/square removal and potassium levels had significant effects on earliness index; whereas seed index was significantly affected by potassium levels (K) and not by branch/square removal. Year mean effect on these parameters was significant with more values recorded during 2012 than 2011. Comparison of treatments' means showed that minimum earliness index (43.93 & 46.15 %) was recorded with removal of all squares from first and second fruiting branch as against maximum earliness index (49.36 & 51. 58%) with no fruiting branch removal. Among potassium rates more earliness index (47.80 & 49.83%) and seed index (7.59 & 8.14 g) were recorded with 150 kg K ha^{-1} compared to other K

Table 4.14: Effect of K level and removal of square/fruiting branch on earliness traits of cotton

	Node number for first fruiting branch		First fruiting branch height (cm)		Earliness index (%)		Seed index (g)	
Square/branch removal (F)	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**
No fruiting branch removal (F_1)	9.00c	7.66c	28.97c	25.57d	49.36a	51.58a	7.14	7.66
Removal of first fruiting branch (F_2)	9.64bc	8.28bc	31.40bc	27.44cd	47.08b	49.18ab	7.22	7.76
Removal of first and second fruiting branch (F_3)	10.08ab	8.73ab	32.75ab	28.73bc	44.81cd	46.70bc	7.38	7.92
Removal of all squares from first fruiting branch (F_4)	10.68a	9.31a	35.22a	30.37ab	46.52bc	48.74b	7.15	7.70
Removal of all squares from first and second fruiting branch (F_5)	10.86a	9.44a	35.64a	30.88a	43.93d	46.15c	7.31	7.84
LSD (5%)	**0.915**	**0.868**	**3.495**	**2.144**	**1.858**	**2.539**	**NS**	**NS**
Potassium level (K)								
50 kg ha^{-1} (K_1)	9.40b	8.06b	30.45b	26.76b	45.03b	47.24b	7.03b	7.55b
100 kg ha^{-1} (K_2)	9.86b	8.48b	32.61b	27.90b	46.19b	48.34ab	7.11b	7.64b
150 kg ha^{-1} (K_3)	10.90a	9.52a	35.33a	31.14a	47.80a	49.83a	7.59a	8.14a
LSD (5%)	**0.708**	**0.672**	**2.707**	**1.660**	**1.439**	**1.967**	**0.266**	**0.454**
Interaction (F × K)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Year mean	10.05a	8.68b	32.80a	28.60b	46.34b	48.47a	7.24b	7.78a
LSD (5%)	**0.388**		**1.433**		**0.968**		**0.254**	

Means not sharing a letter in common differ significantly at 5% probability level.

NS= Non-significant,

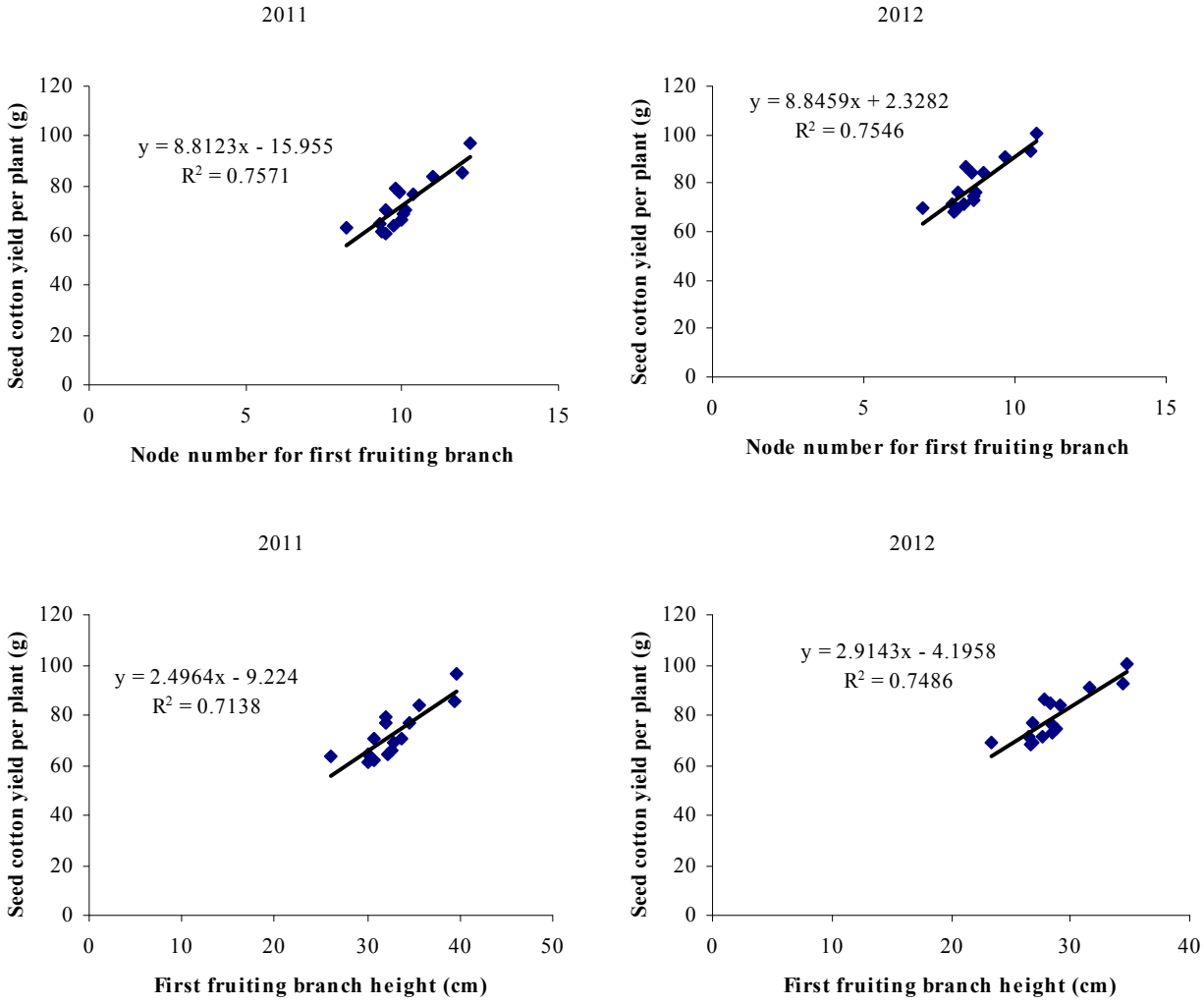


Fig. 4.13: Relationship of node number for first fruiting branch and first fruiting branch height (cm) with seed cotton yield per plant (g)

rates. Relationship of node number for first fruiting branch and first fruiting branch height (cm) with seed cotton yield per plant (g) was strong and positive as shown by linear regression coefficients in figure 4.13.

Senescence of the cotton crop was measured in the form of node above white flower (NAWF). Taking observations on NAWF with weekly interval is a clear measurement of senescence in cotton crop. Periodic data pertaining to nodes above white flower of cotton as affected by fruiting branch/square removal and potassium rates during 2011 and 2012 are depicted in Fig. 4.14 & 4.15. There was visible difference in NAWF of the cotton crop with fruiting branch/square removal and potassium application. In the beginning, more NAWF was observed from 69 to 90 DAS (in 2011) and 68 to 82 DAS (in 2012) thereafter it progressively decreased up to physiologically cut out stage (118 & 117 DAS) when node above white flower reached up to 4. Gradual decrease in NAWF during 2012 was recorded at 82 DAS but in 2011 gradual decrease in NAWF was recorded in 90 DAS. In fruiting branch/square removal more NAWF was observed with removal of all squares from first and second fruiting branch (F_5) followed by F_3 (removal of first and second fruiting branch), F_4 (removal of all squares from first fruiting branch), F_2 (removal of first fruiting branch) and F_1 (no fruiting branch removal) and among potassium application levels highest NAWF was observed with higher potassium dose (150 kg ha^{-1}) than medium (100 kg K ha^{-1}) and lower (50 kg K ha^{-1}) application.

Node above crack boll (NACB) was recorded three times during crop growth. Periodic data of NACB was affected by fruiting branch/square removal and potassium rates (Fig. 4.16 & 4.17). The interactive responses (F x K) were non-significant for NACB. More nodes above cracked bolls were recorded in F_5 (removal of all squares from first and second fruiting branch) and F_3 (removal of first and second fruiting branch) followed by F_2 (removal of first fruiting branch) and F_4 (removal of all squares from first fruiting branch) which were at-par with each other and minimum NACB (nodes above cracked bolls) were observed with no fruiting branch removal. Among potassium rates highest nodes above cracked bolls were recorded with the application of 150 kg K ha^{-1} followed by 100 kg K ha^{-1} and then by 50 kg K ha^{-1}. Periodic data showed that maximum NACB was observed at 132 & 124 DAS which gradually decreased reaching to a minimum of 5 NACB at 174 & 172 DAS during 2011 and 2012, respectively.

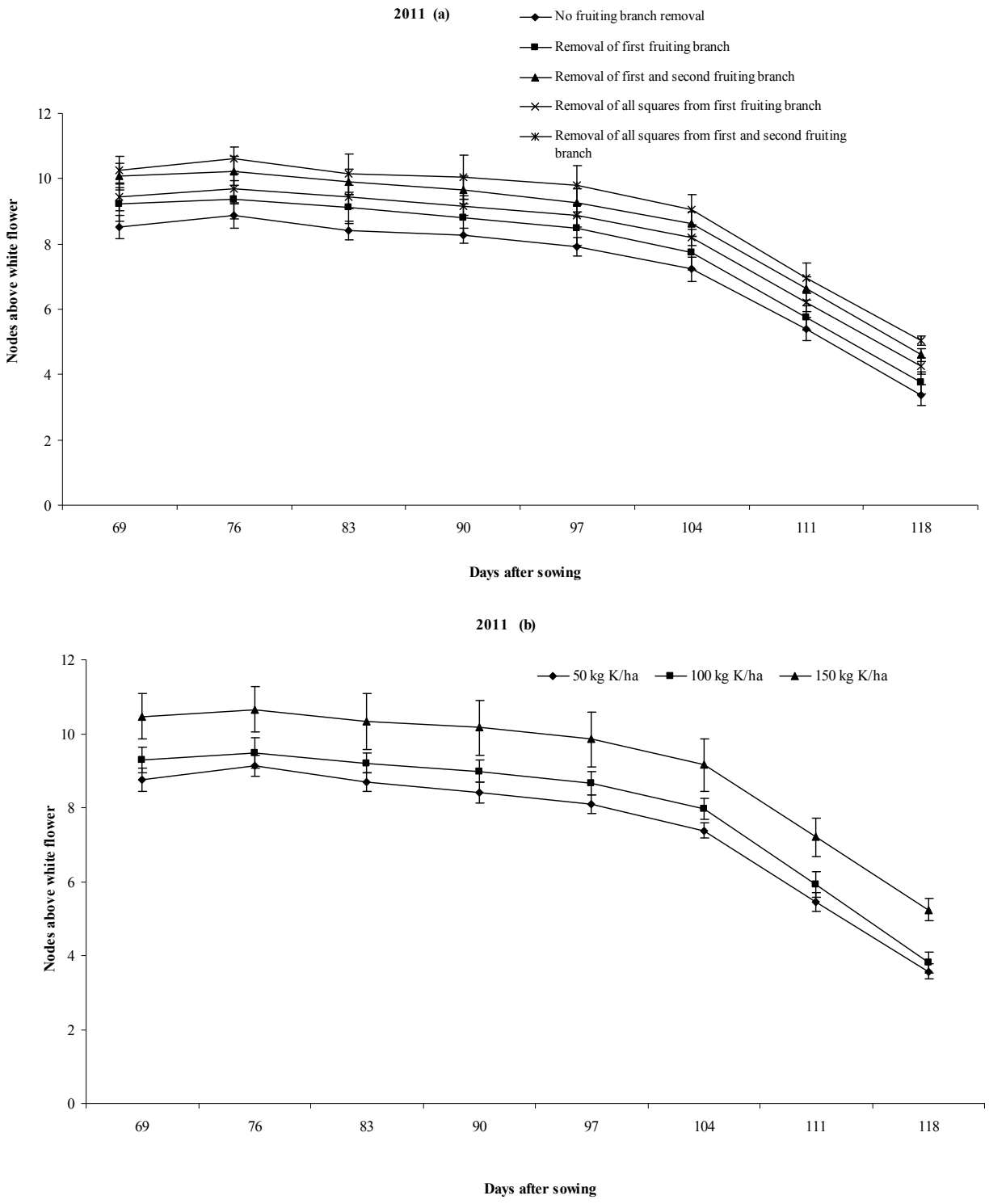
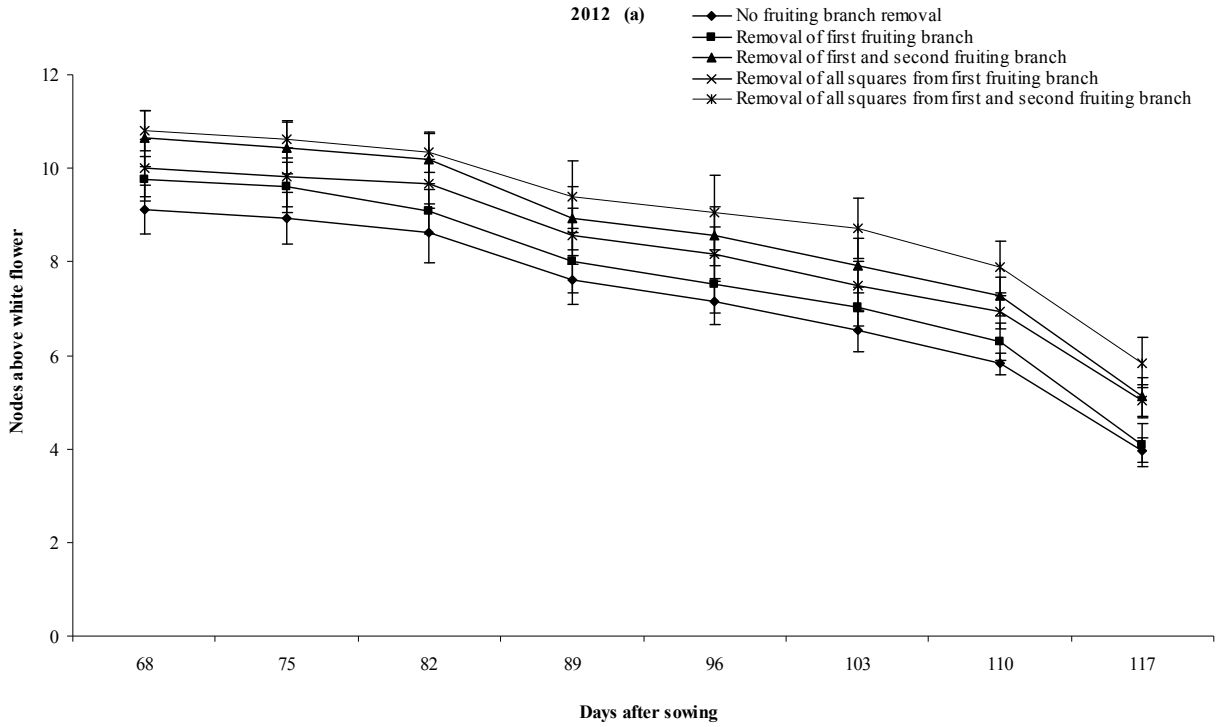


Fig. 4.14: Effect of (a) square/fruiting branch removal and (b) potassium levels on node above white flower in cotton during 2011



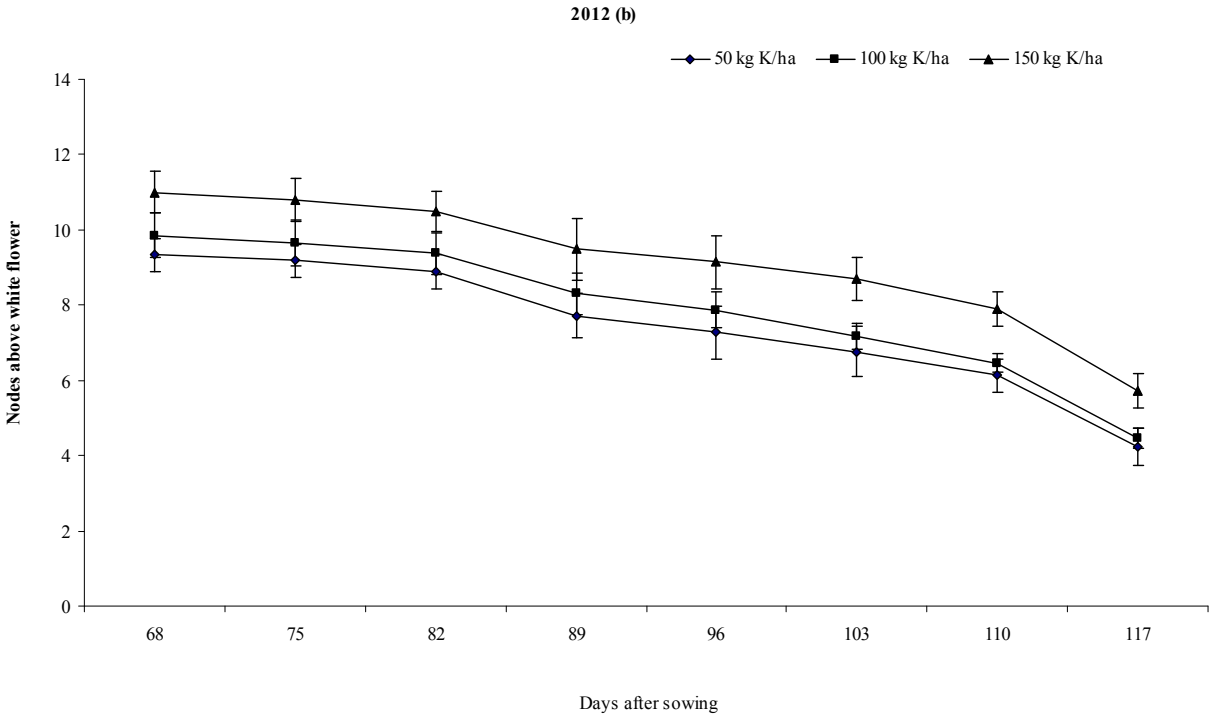


Fig. 4.15: Effect of (a) square/fruiting branch removal and (b) potassium levels on node above white flower in cotton during 2012

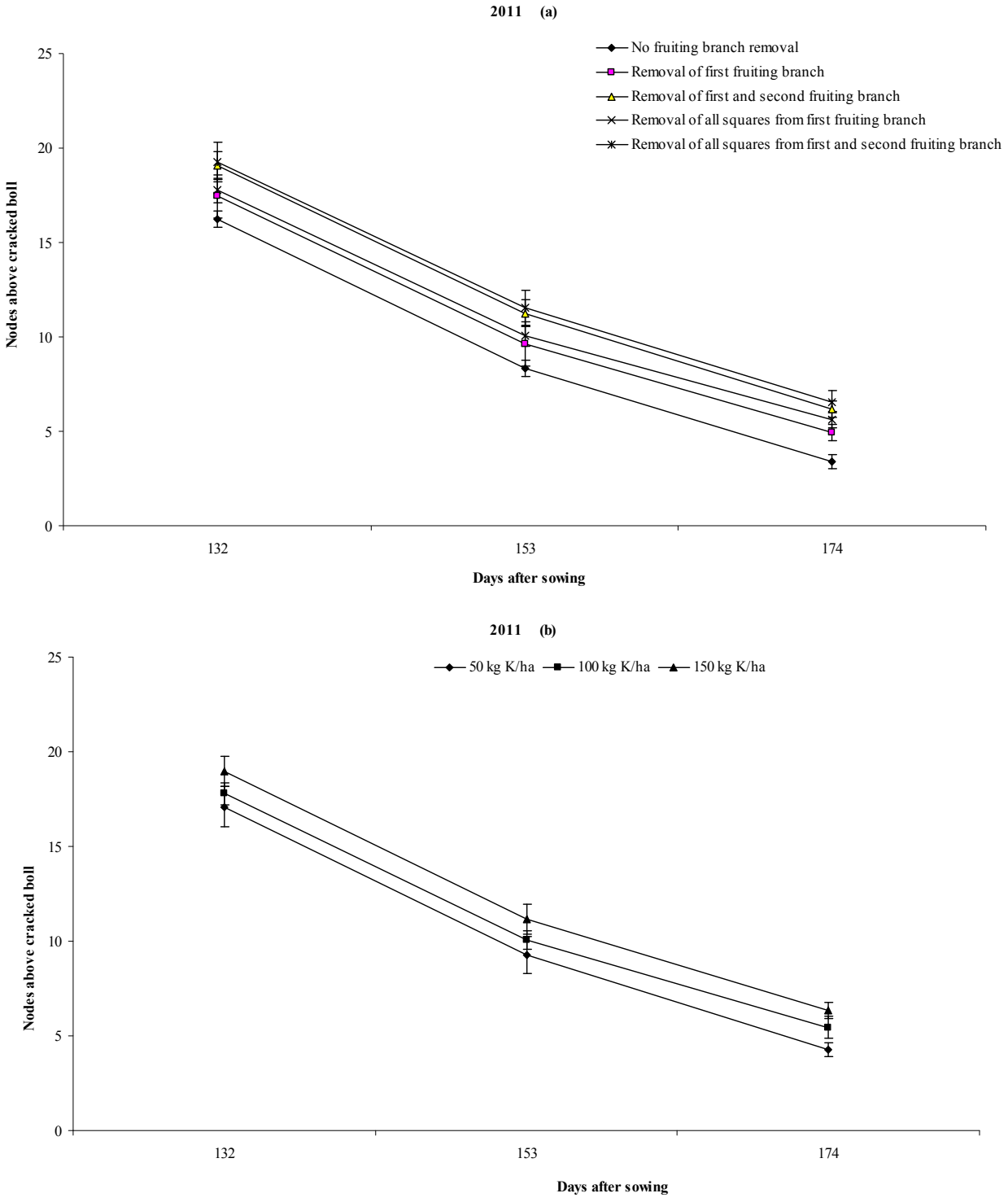


Fig. 4.16: Effect of (a) square/fruiting branch removal and (b) potassium levels on node above cracked boll in cotton during 2011

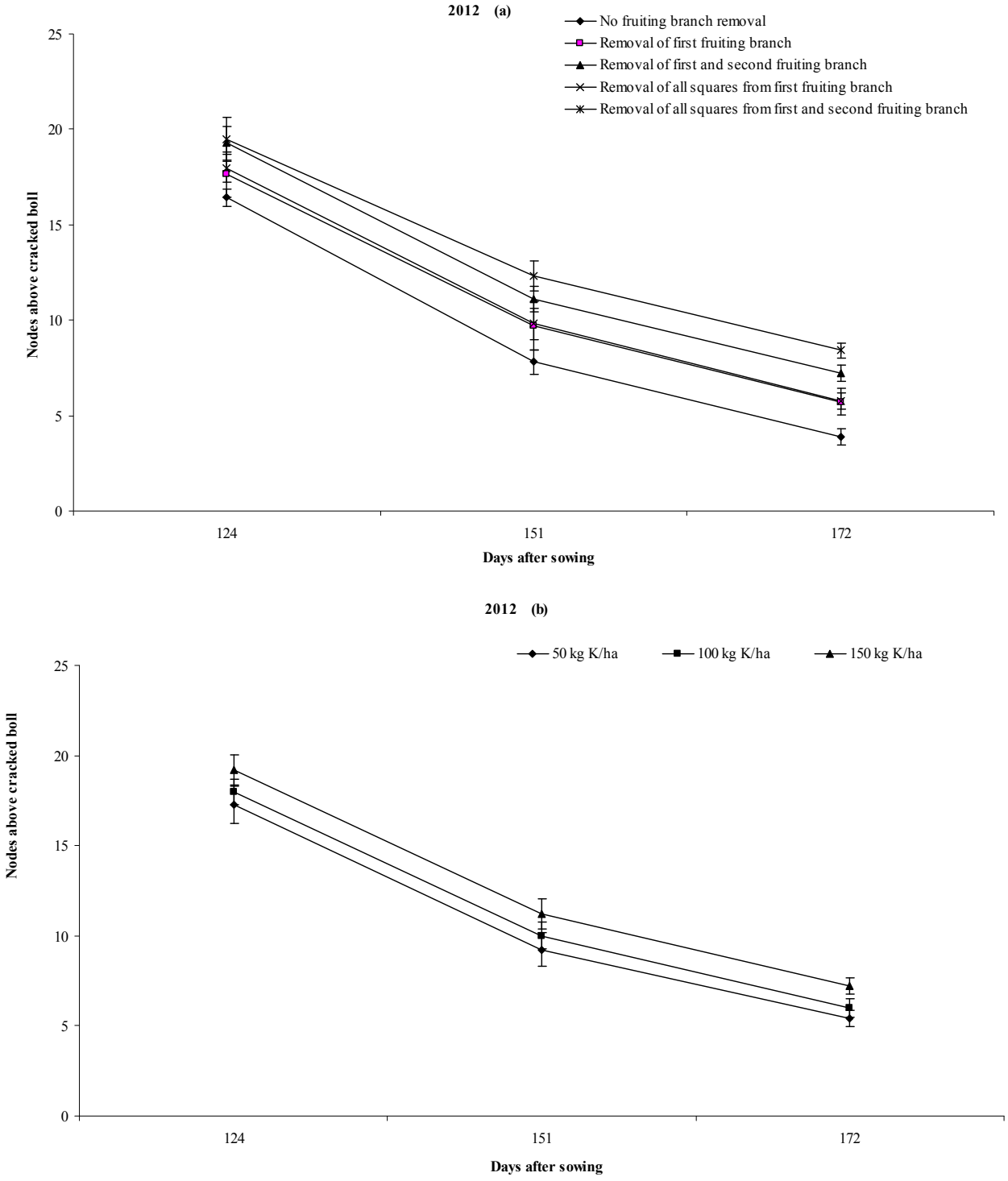


Fig. 4.17: Effect of (a) square/fruiting branch removal and (b) potassium levels on node above cracked boll in cotton during 2012

4.2.4. Discussion

Premature senescence mostly occurring in commercially cultivated Bt cotton might be due to more sink and less source as a result of biological control of boll worm (Dong *et al*., 2006). In addition, senescence is usually associated with increase in ABA and ethylene and decrease in cytokinins (Buchanan, 1997; Oh *et al*., 1997). Among numerous factors such as nutrient deficiency (Wright, 1999), and alteration in phytohormones (Yong *et al*., 2000) especially cytokinins, ABA (Abscisic acid) and ethylene (Yang *et al*., 2004) caused initiation of senescence and its progress in cotton crop. Yield and quality of cotton was affected by both premature senescence and late maturity (Wright, 1999; Dong *et al*., 2006). Node number for first fruiting branch and first fruiting branch height are the morphological measures of earliness in cotton (Joham, 1979). Removal of squares and/or floral buds as well as higher K dose not only delayed senescence but also increased node number for first fruiting branch and first fruiting branch height that may be due to increase in main stem node and increased internodal length. Cotton cultivar matured earlier approximately 4 to 7 days by decrease in one node number of first fruiting branch (Ahmed and Malik, 1996). Shakeel *et al*. (2008) reported that less node number for first fruiting branch is indicator of early senescence in cotton crop. Less boll maturation period with removal of all squares from two early fruiting branches was due to more source availability at early stages which may help in rapid boll filling, but less earliness index in removal of all squares from first and second fruiting branch and with two early fruiting branch removal were due to more sink availability. Potassium deficiency affects crop maturity by stopping reproductive growth prematurely (Pettigrew, 2003). Application of 110 to 250 kg K ha^{-1} is required for growth of cotton and development of fiber (Hodges, 1992). According to Rimon (1989) about 50% potassium is required for boll of total applied potassium and according to Mullins and Burmester (2009) about 24% of applied potassium is required for lint and seed; in our study higher potassium dose reduced the boll maturation period because potassium played significant role in photosynthates translocation from source towards sink (boll). For appropriate management in cotton it is very important to understand causes of senescence and it would help to overcome the losses due to premature and/or late senescence (Dong *et al*., 2009). Cotton gained node above white flower (NAWF < 4) at early stages is an indication of premature senescence (Sarwar *et al.*, 2012). Weekly nodes above white flower were counted from peak flowering till physiological cutout stage to measure its senescence in field condition. In our study

more nodes above white flower at later stages were recorded with removal of all squares from first and second fruiting branch. The removal of early squares might have enhanced the concentration of growth promoting hormones whereas the subtended and main leaves of these branches also served as source of photosynthetic apparatus at initial stages, while minimum nodes above white flower in control treatment may be due to increased concentration of ABA at early stages when square was converted into young boll after fertilization. At peak flowering stage nodes above white flower were more from mid July to first week of August and then gradually decreased so physiological cutout stage came at 2nd week of September in both years of study. Figure 3.1 showed temperature (maximum & minimum) was favorable for cotton growth from July to August during both years of study. Senescence too early (premature senescence) or too late (late maturity) can be measured by nodes above white flower counts (Jones and Snipes, 1999). More nodes above cracked boll were observed in plots, where first and second fruiting branches were removed and supplied with higher dose of potassium, which is an indication of delay in senescence as compared with control.

4.2.5. Agronomic traits

Year mean effect on average boll weight per plant was statistically non-significant but it was significant on number of unopened bolls per plant and number of rotted bolls per plant with more values recorded in 2011 than during 2012. Fruiting branch and/or square removal (F) showed non-significant effect, while potassium rates (K) had significant effect on average boll weight per plant, number of unopened bolls per plant and number of rotted bolls per plant during both years of study. Interactive effect (F x K) was non significant on these parameters. Comparison of treatments' means (table 4.15a) showed that more average boll weight per plant (3.41 & 3.45 g) was recorded in K_3 (150 kg K ha^{-1}) and less average boll weight per plant (3.13 & 3.06 g) was observed with low potassium rate (50 kg K ha^{-1}) that was at-par with medium potassium dose (100 kg K ha^{-1}). Maximum number of unopened bolls per plant (7.28 & 5.97) and minimum rotted bolls per plant (4.54 & 3.09) were recorded with 150 kg K ha^{-1} that was at-par with medium

Table 4.15a: Effect of K level and removal of square/fruiting branch on boll traits of cotton

	Average boll weight per plant (g)		Number of unopened bolls per plant		Number of rotted bolls per plant	
Square/branch removal (F)	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**
No fruiting branch removal (F_1)	3.04	2.98	6.35	5.05	5.02	3.55
Removal of first fruiting branch (F_2)	3.21	3.16	6.71	5.42	5.24	3.86
Removal of first and second fruiting branch (F_3)	3.39	3.32	7.06	5.71	5.62	4.17
Removal of all squares from first fruiting branch (F_4)	3.26	3.23	7.04	5.75	5.02	3.68
Removal of all squares from first and second fruiting branch (F_5)	3.37	3.33	7.22	5.88	5.48	4.00
LSD (p=0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Potassium level (K)						
50 kg ha^{-1} (K_1)	3.13b	3.06b	7.28a	5.97a	4.54b	3.09c
100 kg ha^{-1} (K_2)	3.21ab	3.09b	7.21a	5.86a	4.88b	3.54b
150 kg ha^{-1} (K_3)	3.41a	3.45a	6.14b	4.85b	6.41a	4.93a
LSD (p=0.05)	**0.204**	**0.204**	**0.632**	**0.539**	**0.421**	**0.388**
Interaction (F × N)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Year mean	3.25	3.20	6.88a	5.56b	5.28a	3.85b
LSD (5%)	**NS**		**0.333**		**0.225**	

Means not sharing a letter in common differ significantly at 5% probability level.

NS= Non-significant,

dose (100 kg K ha^{-1}) while less number of unopened bolls per plant (6.14 & 4.85) and more rotted bolls per plant (6.41 & 4.93) was recorded where K was applied at 150 kg K ha^{-1}. Linear regression coefficient (R^2) for average boll weight per plant (g) vs. seed cotton yield per plant (g) was 0.69 & 0.76 during 2011 and 2012, respectively (Fig. 4.18).

Year mean effect on number of insects' damaged bolls per plant was significant however, effect on total number of bolls per plant was non-significant (table 4.15b). Fruiting branch/square removal (F) and potassium rates (K) have significant effects on total number of bolls per plant while number of insects' damaged bolls per plant was affected by potassium dose (K) not by fruiting branch/square removal (F). Interactive effect (F x K) was non significant on these parameters. Comparison of treatments' means showed that maximum insects' damaged bolls per plant (5.09 & 3.84) were recorded with lower potassium dose (50 kg K ha^{-1}) and this damage decreased significantly with increasing potassium (100 or 150 kg K ha^{-1}). More total bolls per plant were recorded with removal of all squares from first and second fruiting branches (F_5) that was at-par with removal of these branches (F_3) and less total number of bolls per plant was recorded in control (F_1); trend was almost similar during both study years (table 4.15b).

Opened bolls per plant were significantly affected by fruiting branch/square removal (F), potassium dose (K), interaction (F x K) and years (table 4.16). More number of opened bolls per plant was recorded in plots of all square/fruiting branch removal treatments and combined higher K dose (150 kg K ha^{-1}); while in control (F_1) all potassium levels performed equally. The trend was same during both study years. Linear regression coefficient (R^2) for opened bolls per plant vs. seed cotton yield per plant (g) was 0.66 & 0.62 for 2011 and 2012, respectively as shown in figure 4.18.

Year mean effect on number of plants attacked by CLCV per plot was non significant during 2011 and 2012. Potassium rate (K) has significant effect on number of plants attacked by CLCV per plot while fruiting branch and/or square removal (F) and their interaction (F x N) had non-significant effect (fig. 4.19). Figure showed that increase in potassium application decreased number of plants attacked by CLCV per plot during both years of study.

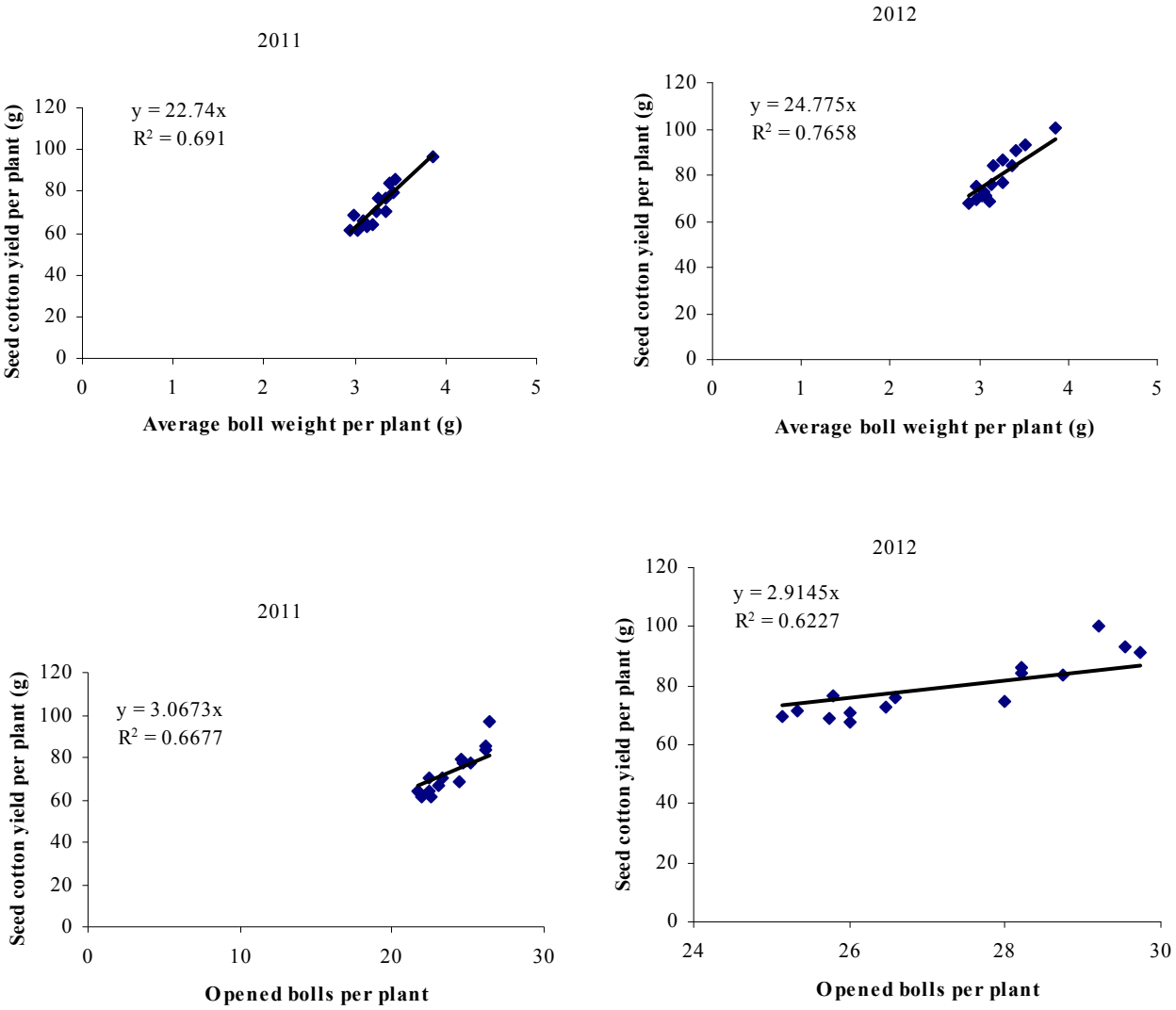


Fig. 4.18: Relationship of average boll weight per plant (g) and opened bolls per plant with seed cotton yield per plant (g)

Table 4.15b: Effect of K level and removal of square/fruiting branch on boll traits of cotton

	Number of insects damaged bolls per plant		Total bolls per plant	
Square/branch removal(F)	**2011**	**2012**	**2011**	**2012**
No fruiting branch removal (F_1)	4.44	3.22	37.91d	37.42d
Removal of first fruiting branch (F_2)	4.64	3.37	39.53cd	39.17c
Removal of first and second fruiting branch (F_3)	4.93	3.66	42.00ab	41.46a
Removal of all squares from first fruiting branch (F_4)	4.60	3.42	40.84bc	40.31b
Removal of all squares from first and second fruiting branch (F_5)	5.13	3.80	43.17a	42.33a
LSD (5%)	**NS**	**NS**	**1.684**	**1.104**
Potassium level (K)				
50 kg ha^{-1} (K_1)	5.09a	3.84a	39.74b	39.18b
100 kg ha^{-1} (K_2)	4.78ab	3.33b	40.33b	39.70b
150 kg ha^{-1} (K_3)	4.37b	3.32b	42.00a	41.53a
LSD (5%)	**0.538**	**0.431**	**1.304**	**0.855**
Interaction (F × K)	**NS**	**NS**	**NS**	**NS**
Year mean	4.75a	3.49b	40.69	40.14
LSD (5%)	**0.271**		**NS**	

Means not sharing a letter in common differ significantly at 5% probability level.

NS= Non-significant,

Table 4.16: Interactive effect of K levels and removal of square/fruiting branch on opened bolls per plant in cotton

	2011	2012
No fruiting branch removal (F_1)		
50 kg ha^{-1} (K_1)	21.80e	25.13e
100 kg ha^{-1} (K_2)	22.53e	26.00e
150 kg ha^{-1} (K_3)	21.93e	25.73e
Removal of first fruiting branch (F_2)		
50 kg ha^{-1} (K_1)	22.40e	26.00e
100 kg ha^{-1} (K_2)	21.73e	25.33e
150 kg ha^{-1} (K_3)	24.66bcd	28.20abc
Removal of first and second fruiting branch (F_3)		
50 kg ha^{-1} (K_1)	22.46e	25.80e
100 kg ha^{-1} (K_2)	24.53bcd	28.20abc
150 kg ha^{-1} (K_3)	26.13abc	29.73a
Removal of all squares from first fruiting branch (F_4)		
50 kg ha^{-1} (K_1)	23.00de	26.46de
100 kg ha^{-1} (K_2)	23.33de	26.60cde
150 kg ha^{-1} (K_3)	26.20ab	29.53ab
Removal of all squares from first and second fruiting branch (F_5)		
50 kg ha^{-1} (K_1)	24.46cd	28.00bcd
100 kg ha^{-1} (K_2)	25.13abc	28.73ab
150 kg ha^{-1} (K_3)	26.40a	29.20ab
LSD 5 %	**1.689**	**1.699**
Year mean	23.78b	27.24a
LSD 5 %	**0.427**	

Means not sharing a letter in common differ significantly at 5% probability level.

NS= Non-significant,

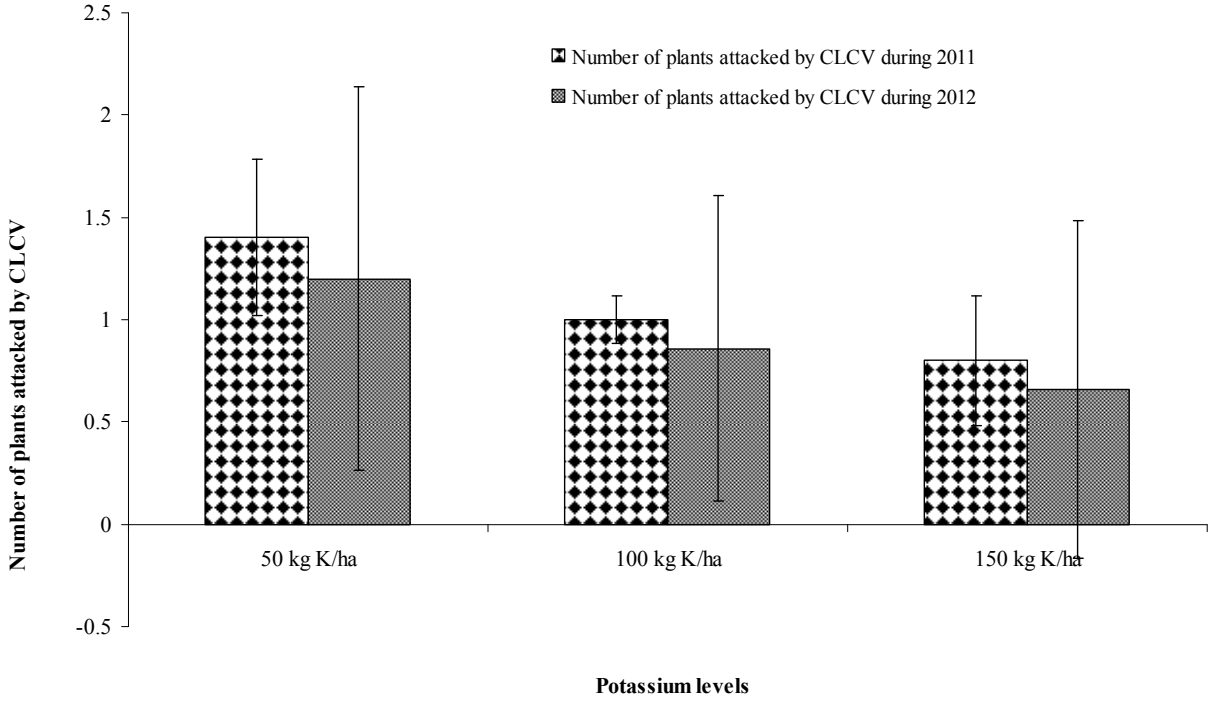


Fig. 4.19: Effect of potassium levels on number of plants attacked by CLCV in cotton

Year mean effect on number of monopodial and sympodial branches per plant was significant showing more values in 2012 than 2011 (table 4.17). Treatments' means and interaction showed significant effect on number of monopodial branches and number of sympodial branches per plant. Maximum number of monopodial branches per plant (2.66 & 3.13) was recorded with removal of all squares from first and second fruiting branch at higher potassium application (150 kg ha^{-1}) that was at par with medium dose (100 kg K ha^{-1}) of potassium application. Same trend was observed in F_3 (removal of first and second fruiting branch) with higher, medium and low potassium rate; while in removal of first fruiting branch (F_2) and removal of all squares from this branch (F_4) higher dose of K produced significantly more branches than other two levels of potassium. Although increasing K also increased number of monopodial branches in control (no fruiting branch removal) however, its performance remained at bottom than all fruiting branch removal treatments (table 4.17). For number of sympodial branches all treatments of square or branch removal performed best at higher potassium dose than at medium or lower levels however, in control (no square/branch removal) all three potassium levels were at par (P=0.05) with each other. Same trend was followed during both study years (table 4.17). Figure 4.20 showed strong positive relationship for monopodial and sympodial branches per plant with seed cotton yield per plant (g).

Year mean effect on seed cotton yield (per plant and per hectare) was significant. It was more in 2012 than 2011. Data pertaining to seed cotton yield as influenced by treatments' means as well as interaction (F x K) were significantly difference (table 4.18). Maximum seed cotton yield was recorded in plots where all squares were removed from first and second fruiting branches and cotton plants were supplied with higher K dose (150 kg K ha^{-1}) during both years of study. Application of F_3 (removal of first and second fruiting branch) and F_4 (removal of all squares from fruiting branch) treatments also performed well at higher K dose with respect to seed cotton yield. Trend for K application dose was also similar in F_2 (removal of first fruiting branch) but its performance was poor than other treatments of branch or square removal. While in control (no fruiting branch removal) all the three potassium rates were statistically (P=0.05) same for the parameter under discussion. The trend was same during both study years. Value for R^2 (Fig. 4.22) showed strong and positive association of seed cotton yield per plant (g) with seed cotton yield (kg) per hectare.

Table 4.17: Interactive effect of K levels and removal of square/fruiting branch on number of branches in cotton

	Monopodial branches per plant		Sympodial branches per plant	
	2011	**2012**	**2011**	**2012**
No fruiting branch removal (F_1)				
50 kg ha^{-1} (K_1)	1.20e	1.73fg	17.73ef	18.73de
100 kg ha^{-1} (K_2)	1.13e	1.53g	18.00ef	19.13de
150 kg ha^{-1} (K_3)	1.53d	1.86f	18.93de	19.93cd
Removal of first fruiting branch (F_2)				
50 kg ha^{-1} (K_1)	1.20e	1.86f	17.40f	18.60de
100 kg ha^{-1} (K_2)	1.26e	1.73fg	18.13ef	19.33de
150 kg ha^{-1} (K_3)	1.66d	2.20de	20.80bc	22.06ab
Removal of first and second fruiting branch (F_3)				
50 kg ha^{-1} (K_1)	1.73cd	2.26cde	17.20f	18.80de
100 kg ha^{-1} (K_2)	1.93bc	2.46bcd	20.06cd	21.20bc
150 kg ha^{-1} (K_3)	2.13b	2.73b	22.13a	23.20a
Removal of all squares from first fruiting branch (F_4)				
50 kg ha^{-1} (K_1)	1.53d	2.00ef	17.60f	18.53e
100 kg ha^{-1} (K_2)	1.66d	2.20de	18.40ef	19.33de
150 kg ha^{-1} (K_3)	2.00b	2.53bc	21.40ab	22.46ab
Removal of all squares from first and second fruiting branch (F_5)				
50 kg ha^{-1} (K_1)	1.93bc	2.46bcd	18.33ef	19.26de
100 kg ha^{-1} (K_2)	2.60a	3.13a	20.20bc	21.60b
150 kg ha^{-1} (K_3)	2.66a	3.13a	22.40a	23.40a
LSD 5 %	**0.262**	**0.295**	**1.208**	**1.343**
Year mean	1.74b	2.25a	19.24b	20.37a
LSD 5 %	**0.071**		**0.334**	

Means not sharing a letter in common differ significantly at 5% probability level.

NS= Non-significant,

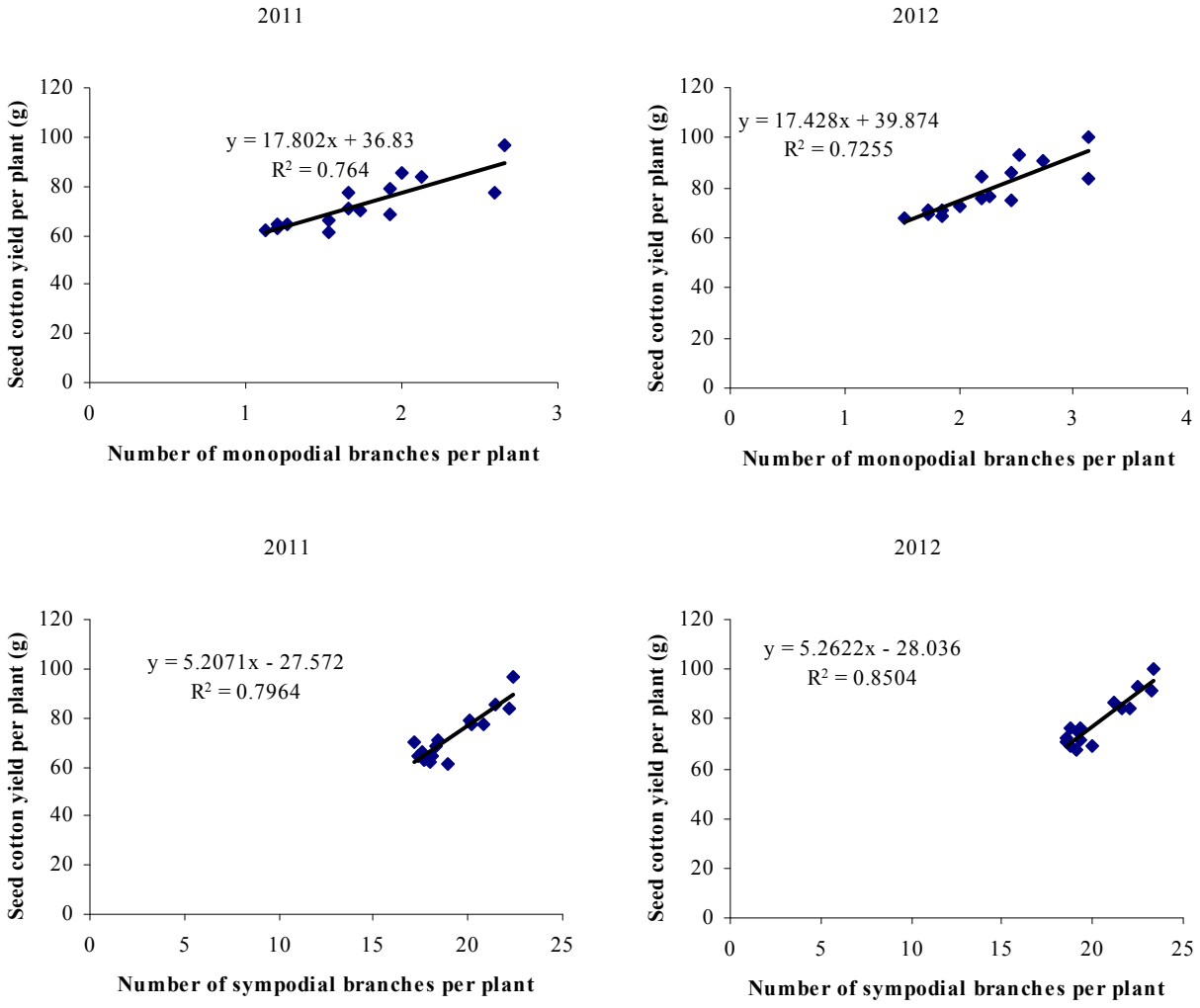


Fig. 4.20: Relationship of number of monopodial and sympodial branches per plant with seed cotton yield per plant (g)

Table 4.18: Interactive effect of K levels and removal of square/fruiting branch on seed cotton yield

	Seed cotton yield per plant (g)		Seed cotton yield (kg ha^{-1})	
	2011	**2012**	**2011**	**2012**
No fruiting branch removal (F_1)				
50 kg ha^{-1} (K_1)	63.20e	69.37e	2677f	2819i
100 kg ha^{-1} (K_2)	61.66e	67.93e	2763ef	3004hi
150 kg ha^{-1} (K_3)	61.13e	68.87e	2773def	3063ghi
Removal of first fruiting branch (F_2)				
50 kg ha^{-1} (K_1)	64.40e	71.07e	2884def	3164fgh
100 kg ha^{-1} (K_2)	64.33e	71.23e	3114cde	3286efgh
150 kg ha^{-1} (K_3)	77.06bc	84.47bcd	3448bc	3755bc
Removal of first and second fruiting branch (F_3)				
50 kg ha^{-1} (K_1)	70.33cde	76.53cde	3150cde	3402def
100 kg ha^{-1} (K_2)	79.13bc	86.40bc	3402bc	3498cde
150 kg ha^{-1} (K_3)	83.73b	91.00ab	4056a	4246a
Removal of all squares from first fruiting branch (F_4)				
50 kg ha^{-1} (K_1)	66.26de	72.70e	3166cd	3233efgh
100 kg ha^{-1} (K_2)	70.53cde	75.97de	3090cde	3296efgh
150 kg ha^{-1} (K_3)	85.60b	92.93ab	3642b	3871b
Removal of all squares from first and second fruiting branch (F_5)				
50 kg ha^{-1} (K_1)	68.73cde	74.70de	3060cdef	3323efg
100 kg ha^{-1} (K_2)	76.93bcd	83.93bcd	3641b	3690bcd
150 kg ha^{-1} (K_3)	96.73a	100.27a	4146a	4328a
LSD 5 %	**10.671**	**9.996**	**393.80**	**316.80**
Year mean	72.65b	79.15a	3267	3465
LSD 5 %	**2.580**		**88.70**	

Means not sharing a letter in common differ significantly at 5% probability level.

NS= Non-significant,

Plant height remained non-significant at squaring stage (table 4.19), however at physiological cutout stage and at last pick it was significantly affected by fruiting branch and/or square removal, potassium rates and their interaction during both years of study. Interactive effect (fig. 4.21) showed that cotton plants gained more height when we removed all squares from first and second fruiting branch (F_5) or when first and second fruiting branches were removed (F_3) at highest level of K application. Trend was almost similar during both years of study. There was strong positive relationship ($R^2 > 0.80$) between plant height (cm) at last pick with seed cotton yield per plant (g) as shown in fig. 4.22.

4.2.6. Discussion

Potassium is the mineral element required in higher quantity by cotton (Oosterhuis *et al*., 2013). For optimal cotton growth 2 to 5% K is required on dry weight basis (Marschner, 1995). Unnecessary K application in cotton enhanced plant height (Pettigrew and Meredith, 1997) and postponed maturity (Clement and Gwathmey, 2007; Gwathmey *et al*., 2009); while deficiency of K decreased leaf area index, plant biomass and photosynthesis (Hezhong *et al*., 2004), lint percentage (Pettigrew *et al*., 1996), lint yield (Read *et al*., 2006), dry matter production (Rosolem *et al*., 2003), plant height (Zhao *et al*., 2001), internodal length, leaf area (Gerardeaux *et al*., 2010), seed & boll mass (Pettigrew *et al*., 1996) and nitrogen uptake (Pettigrew and Meredith, 1997). Square removal and leaf cut treatments were applied on Bt cotton; square removal increased boll size and reduced insecticidal protein content but leaf cut increased Bt toxin content and decreased boll size (Yonghui *et al*., 2009). Result of two years field trial on Bt cotton showed that two basal fruiting branches removal at squaring significantly increased lint yield (5.2 to 7.5 %), boll size (5.1 to 5.7 %), number of fruiting nodes, Cry1Ac protein in the fully expanded young leaves and Cry1Ac expression in terms of more insect pests resistance compared with their control treatment (Dong *et al*., 2008). From agronomic point of view, deficiency and excess of potassium are environmentally and economically unproductive and have harmful impact on yield of cotton (Oosterhuis *et al*., 2013). In our study less number of opened bolls during 2011 may be due to more rainfall at early season which caused more young boll loss at initial stages that might be the possible reason of lower seed cotton yield per plant during 2011 than 2012 (Fig. 3.1).

Table 4.19: Effect of K level and removal of square/fruiting branch on plant height (cm) at appearance of first floral bud

	2011	2012
Square/branch removal (F)		
No fruiting branch removal (F_1)	32.68	34.82
Removal of first fruiting branch (F_2)	32.62	34.66
Removal of first and second fruiting branch (F_3)	33.51	35.40
Removal of all squares from first fruiting branch (F_4)	33.24	35.26
Removal of all squares from first and second fruiting branch (F_5)	34.24	36.31
LSD (5%)	**NS**	**NS**
Potassium level (K)		
50 kg ha^{-1} (K_1)	32.26	34.24
100 kg ha^{-1} (K_2)	33.60	35.61
150 kg ha^{-1} (K_3)	33.92	36.02
LSD (5%)	**NS**	**NS**
Interaction (F × K)	**NS**	**NS**
Year mean	33.26b	35.29a
LSD (5%)	**2.011**	

Means not sharing a letter in common differ significantly at 5% probability level.

NS= Non-significant,

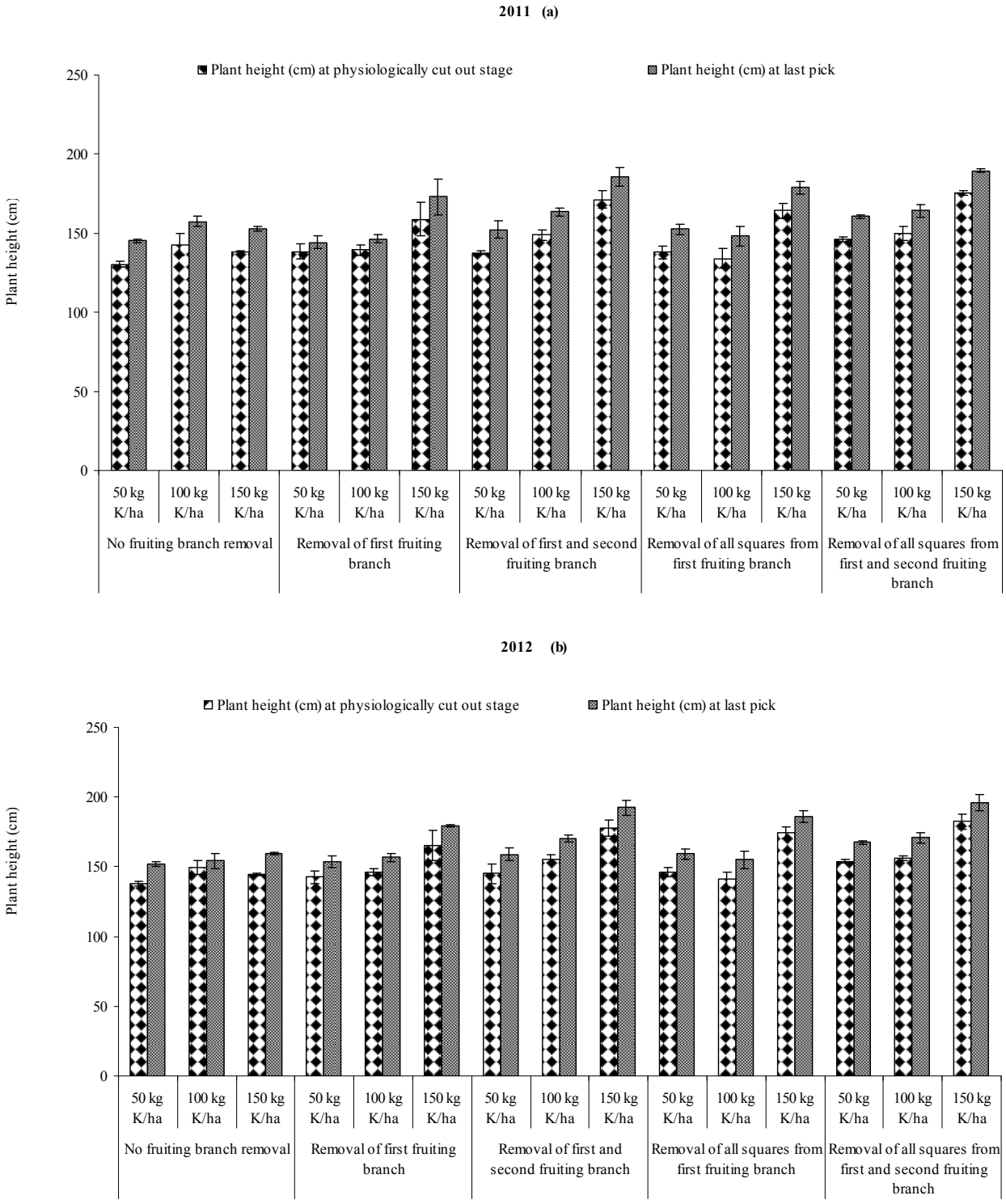


Fig. 4.21: Interactive effect of K levels and removal of squares and/or fruiting branch on plant height of cotton

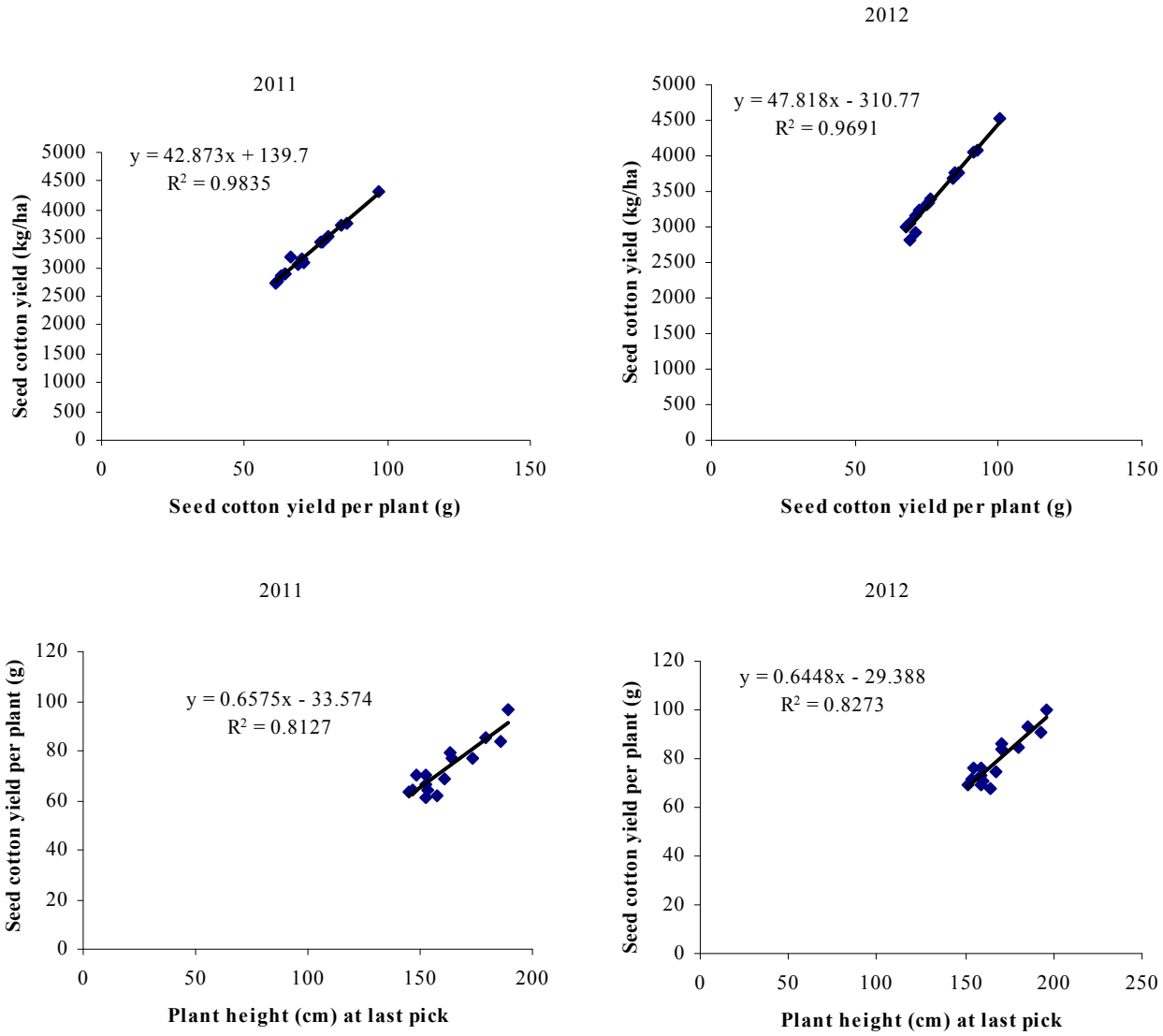


Fig. 4.22: Relationship between seed cotton yield per plant (g) vs. seed cotton yield per hectare (kg) and plant height (cm) last pick vs. seed cotton yield per plant (g)

Potassium plays a vital role in plants resistance and tolerance to pathogens (Hezhong *et al*., 2004). In our study increase in potassium dose reduced number of plants attacked by CLCV. Potassium perhaps induced its chief influences on disease through precise metabolic functions that modify compatibility associations of the host-parasite surroundings (Kafkafi *et al*., 2001).

Number of monopodial and sympodial branches are important yield determining traits in cotton. Increase in number of monopodial and sympodial branches with removal of squares from first and second fruiting branch or with removal of first and second fruiting branch was due to increase in main stem nodes by increasing plant height; manual removal of early squares increased the concentration of cytokinins and decreased concentration of abscisic acid in cotton and its effect remained effective till 45 days after the removal (Dong *et al*., 2009). Square and/or branch removal interacts with potassium and increased more vegetative growth at initial stages leading to more number of main stem nodes that may be responsible for more number of monopodial and sympodial branches per plant. Earlier findings showed that two basal fruiting branches removal at squaring increased plant height, leaf area and plant biomass as compared to control (Dong *et al*., 2008). We assumed that square and/or fruiting branches removal at early stage decreased sink/source ratio; less sink at early stage improved more vegetative growth (increased more source) that may be helpful at later stages for boll filling. Previous results showed that flower-bud removal can also increase single-leaf as well as canopy photosynthesis rate (Dumka *et al*., 2003); subtended leaf has 60 % role in boll filling that led to increased seed cotton yield per plant (g) and seed cotton yield per hectare in our study. Less seed cotton yield during 2011 may be due to more rain fall at initial stages of crop growth that led to shedding of more flowers at initial stages causing less bolls to contribute seed cotton yield (Fig. 3.1). Increase in total number of bolls per plant may be due to more number of sympodial and monopodial branches per plant. Results of field and pot trials showed that more bolls and highest seed cotton yield were recorded with application of 225 kg K ha^{-1} (Zheng *et al*., 2013). At squaring, differences in plant height were non-significant. Thereafter delay in senescence (increased plant height) was recorded in plots where either two early fruiting branches were removed or where all squares were removed from those two fruiting branches. The previous studies showed that fruit loss changes the partitioning of plant resources in support of vegetative growth (Sadras 1995; Jones *et al*., 1996). Preferred partitioning of photosynthates towards vegetative parts like root, stem, and leaf due to fruit losses might be responsible for the increased plant height (Sadras,

1996). Early fruit (sink) removal enhanced the vegetative growth and increased fruiting from later-developed positions also compensated earlier losses of fruit (Bednarz and Roberts 2001).

4.2.7. Quality traits

Year mean effect was significant on ginning out turn (%), fiber length (mm), fiber strength (g tex^{-1}), fiber fineness (micronaire), fiber elongation (%), seed protein content (%) and seed oil content (%) and non significant on fiber uniformity (%), with more values recorded in 2012 than 2011 except seed protein content (%) which showed opposite trend (table 4.20a and 4.20b).

Fruiting branch and/or square removal (F) showed non-significant effect on fiber strength, fineness, uniformity and elongation however, ginning out turn, fiber length, seed protein content and seed oil content were significantly affected by fruiting branch and/or square removal. Effect of potassium rate (K) was significant while interactive effect (F x K) was non-significant on all above mentioned quality traits. Comparison of treatments' means (table 4.20a) showed more ginning out turn (GOT) in F_5 (removal of all squares from first and second fruiting branch) and F_3 (removal of first and second fruiting branch) being at par with each other but significantly better than F_1 (no fruiting branch removal), F_2 (removal of first fruiting branch) and F_4 (removal of all squares from first fruiting branch). Maximum fiber length (26.21 & 27.38 mm) was recorded in removal of first and second fruiting branch (F_3) that was at-par with F_5 (removal of all squares from first and second fruiting branch) and minimum fiber length (24.92 & 26.12 mm) was observed in control (F_1). The data given in table 4.20b show that removal of all squares from first and second fruiting branch (F_5) and removal of first and second fruiting branch (F_3) increased seed protein and seed oil content to a significant level than all other treatments during both years. Among potassium rates, increasing potassium increased values for all quality traits under discussion (table 4.20a, b).

4.2.8. Discussion

Overall objective of cotton production is lint production; increased lint yield indirectly enhanced ginning out turn (GOT). According to an estimate three percent increase in seed cotton yield could be expected with one percent increase in ginning out turn (Saleem *et al*., 2010b). Our experimental results also depicted positive relationship (R^2= 0.75 & 0.77) between ginning out

Table 4.20a: Effect of K level and removal of square/fruiting branch on quality traits of cotton

	Ginning out turn (%)		Fiber length (mm)		Fiber strength (g tex^{-1})		Fiber fineness (Micronaire)	
Square/branch removal (F)	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**
No fruiting branch removal (F_1)	36.92c	38.01c	24.92c	26.12c	22.46	23.15	5.36	5.78
Removal of first fruiting branch (F_2)	38.04bc	39.13bc	25.26bc	26.48bc	22.86	23.51	5.30	5.73
Removal of first and second fruiting branch (F_3)	39.92a	41.01a	26.21a	27.38a	22.71	23.71	5.52	5.96
Removal of all squares from first fruiting branch (F_4)	38.43b	39.51b	25.61ab	26.66bc	22.91	23.24	5.34	5.75
Removal of all squares from first and second fruiting branch (F_5)	39.76a	40.85a	25.78ab	26.97ab	22.30	23.73	5.37	5.84
LSD (5%)	**1.146**	**1.150**	**0.637**	**0.627**	**NS**	**NS**	**NS**	**NS**
Potassium level (K)								
50 kg ha^{-1} (K_1)	37.40c	38.49c	24.93c	26.14c	21.52c	22.33c	5.60a	6.03a
100 kg ha^{-1} (K_2)	38.49b	39.58b	25.48b	26.66b	22.72b	23.54b	5.26b	5.73b
150 kg ha^{-1} (K_3)	39.95a	41.03a	26.26a	27.38a	23.70a	24.53a	5.28b	5.68b
LSD (5%)	**0.888**	**0.891**	**0.493**	**0.486**	**0.875**	**0.877**	**0.274**	**0.252**
Interaction (F × K)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Year mean	38.61b	39.70a	25.56b	26.72a	22.65b	23.47a	5.38b	5.81a
LSD (5%)	**0.511**		**0.272**		**0.504**		**0.189**	

Means not sharing a letter in common differ significantly at 5% probability level.

NS= Non-significant,

Table 4.20b: Effect of K level and removal of square/fruiting branch on quality traits of cotton

	Fiber uniformity (%)		Fiber elongation (%)		Seed protein content (%)		Seed oil content (%)	
Square/branch removal (F)	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**
No fruiting branch removal (F_1)	48.31	48.42	11.18	11.61	13.60b	12.39c	14.27c	15.33c
Removal of first fruiting branch (F_2)	49.16	49.30	11.63	12.11	15.31ab	13.36c	16.33b	17.38bc
Removal of first and second fruiting branch (F_3)	46.83	47.00	11.44	11.92	17.25a	15.55ab	18.33a	19.66a
Removal of all squares from first fruiting branch (F_4)	49.30	49.38	11.37	11.80	15.31ab	14.09bc	16.44b	17.61ab
Removal of all squares from first and second fruiting branch (F_5)	48.43	48.62	12.05	12.50	17.25a	17.01a	18.38a	19.61ab
LSD (5%)	**NS**	**NS**	**NS**	**NS**	**2.035**	**2.116**	**1.695**	**2.267**
Potassium level (K)								
50 kg ha^{-1} (K_1)	47.09b	47.23b	11.10b	11.53b	14.87b	12.68b	14.13b	15.16b
100 kg ha^{-1} (K_2)	47.71b	47.86b	11.47b	11.94ab	15.16b	13.55b	17.50a	18.73a
150 kg ha^{-1} (K_3)	50.42a	50.54a	12.04a	12.48a	17.20a	17.20a	18.63a	19.86a
LSD (5%)	**1.601**	**1.598**	**0.563**	**0.553**	**1.577**	**1.639**	**1.313**	**1.756**
Interaction (F × K)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Year mean	48.40	48.54	11.54b	11.98a	15.74a	14.48b	16.75	17.92
LSD (5%)	**NS**		**0.318**		**0.947**		**0.974**	

Means not sharing a letter in common differ significantly at 5% probability level.

NS= Non-significant,

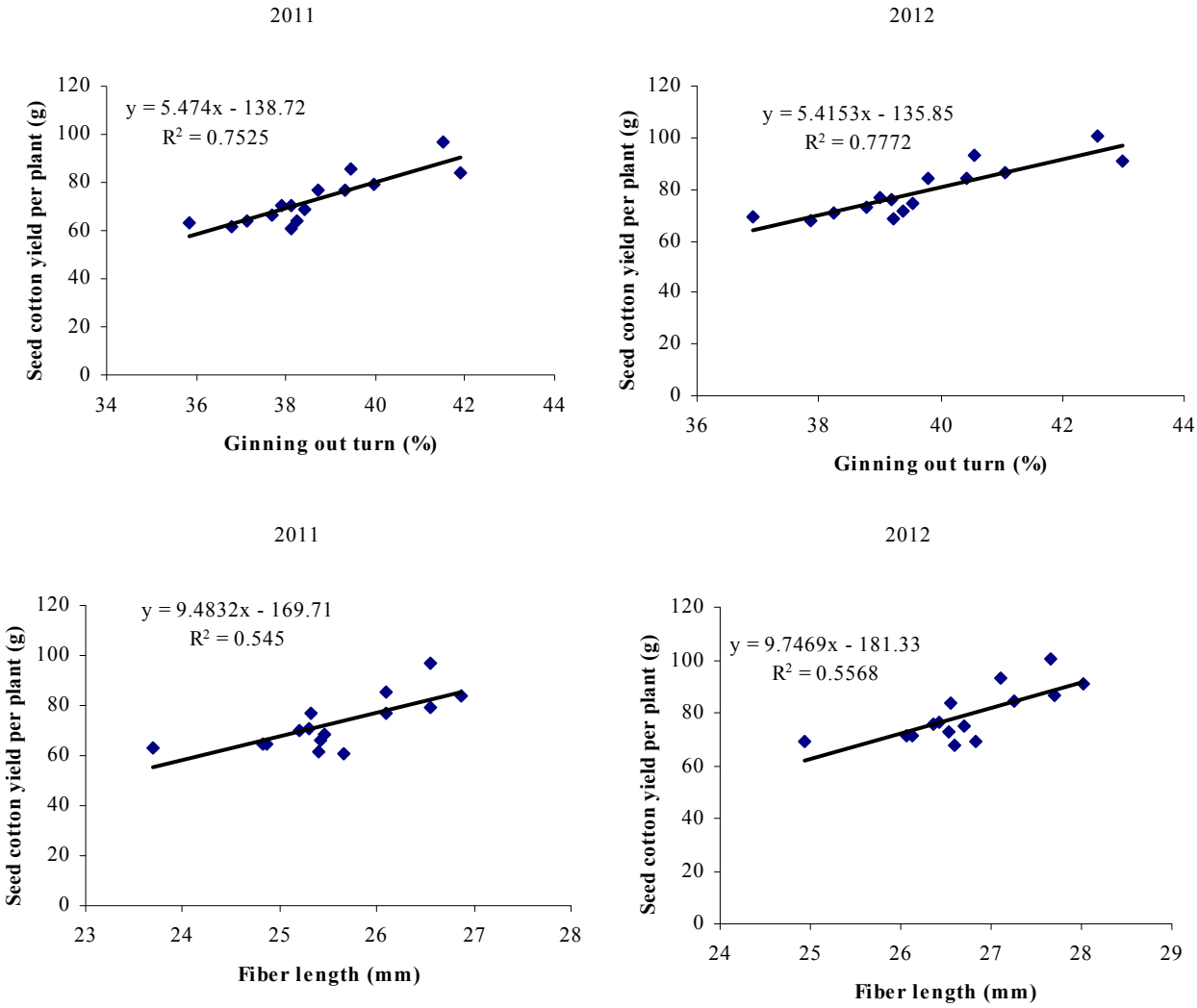


Fig. 4.23: Relationship of ginning out turn (%) and fiber length (mm) with seed cotton yield per plant (g)

turn and seed cotton yield per plant (g) as shown in figure 4.23. The relationship between fiber length and seed cotton yield per plant (fig. 4.23) was also reasonably good (R^2= 0.54 and 0.55). Yarn spinning ability depends on fiber strength; cotton with low strength or weak fiber is difficult to handle in manufacturing process. Oosterhuis (1994) reported that potassium has the ability to produce turgor pressure which makes the fiber to elongate more. If K is deficient during fiber formation, it will cause reduction in turgor pressure which ultimately causes shorter length of fiber. Application of K increased fiber micronaire by one percent and fiber elongation by three percent; application of K had no effect on other characteristics of fiber (Pettigrew *et al*., 2005). Application of 250 kg K ha^{-1} improved fiber strength, fineness, maturity ratio and length; also positive correlation was found among quality traits of fiber and K contents of leaf tissues at bloom stage (Pervez *et al*., 2004).

4.2.9. Biochemical traits

Data (table 4.21) pertaining to nitrogen concentration (%) in cotton leaf was significantly affected by fruiting branch and/or square removal and potassium doses while their interaction (F x K) was non-significant. Year effect was significant with more nitrogen content (%) during 2011 than 2012. Maximum nitrogen concentration (2.80 & 2.60%) in cotton leaf was observed with removal of all squares from first and second fruiting branch (F_5) that was at-par with removal of first and second fruiting branch (F_3) while minimum nitrogen concentration (2.02 & 1.78%) in leaf was observed in control (no fruiting branch removal). Among potassium rates high nitrogen concentration (2.63 & 2.40%) in cotton leaf was recorded with higher potassium application (150 kg K ha^{-1}) while low nitrogen concentration (2.24 & 1.93%) in cotton leaf was recorded with the application of 50 kg K ha^{-1} (low K rate) during 2011 & 2012. Linear regression coefficient (R^2) for nitrogen concentration (%) in cotton leaf vs. seed cotton yield per plant (g) was good enough as shown in figure 4.24.

Data in table 4.22 indicated that year means were significantly different in potassium concentration (mg g^{-1}) with more values recorded in 2011 than during 2012. Interactive effect showed significant increase in potassium concentration (mg g^{-1}) in cotton leaf when we removed all squares from first and second fruiting branch (F_5) or with removal of first and second fruiting branch (F_3) and plants were supplied with 150 kg K ha^{-1}. This was followed by F_4 (removal of all squares from first fruiting branch), F_2 (removal of first fruiting branch) and then F_1 (no fruiting

Table 4.21: Effect of K level and removal of square/fruiting branch on N concentration (%) in cotton leaf

	Leaf N %	
Square/branch removal (F)	**2011**	**2012**
No fruiting branch removal (F_1)	2.02c	1.78c
Removal of first fruiting branch (F_2)	2.29b	2.02b
Removal of first and second fruiting branch (F_3)	2.72a	2.41a
Removal of all squares from first fruiting branch (F_4)	2.33b	2.06b
Removal of all squares from first and second fruiting branch (F_5)	2.80a	2.60a
LSD (5%)	**0.254**	**0.218**
Potassium level (K)		
50 kg ha^{-1} (K_1)	2.24b	1.93c
100 kg ha^{-1} (K_2)	2.42b	2.19b
150 kg ha^{-1} (K_3)	2.63a	2.40a
LSD (5%)	**0.196**	**0.169**
Interaction (F × K)	**NS**	**NS**
Year mean	2.43a	2.17b
LSD (5%)	**0.113**	

Means not sharing a letter in common differ significantly at 5% probability level.

NS= Non-significant,

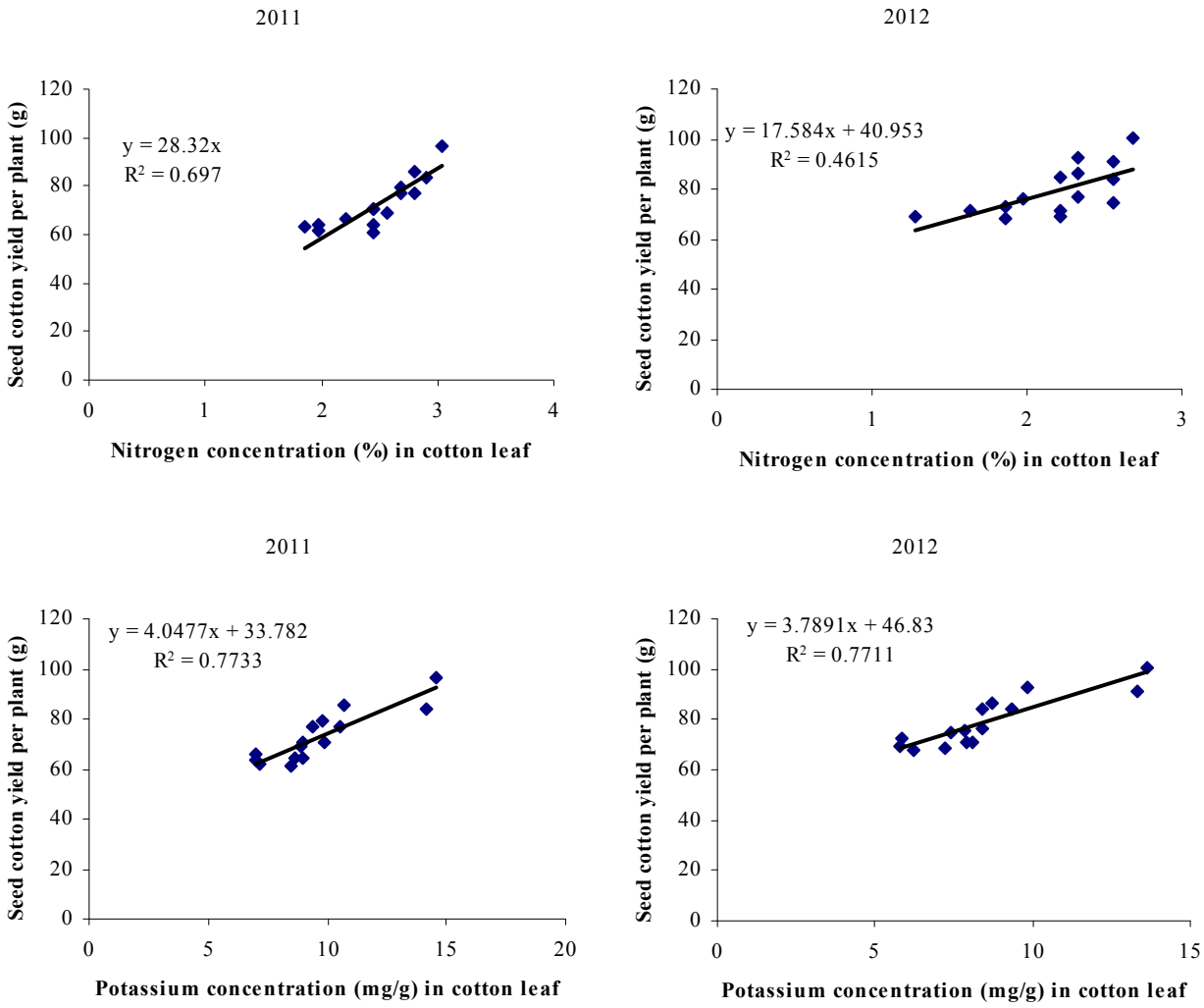


Fig. 4.24: Relationship of nitrogen concentration (%) and potassium concentration (mg/g) in cotton leaf with seed cotton yield per plant (g)

Table 4.22: Interactive effect of K levels and removal of square/fruiting branch on K concentration (mg g^{-1}) in cotton leaf

	2011	2012
No fruiting branch removal (F_1)		
50 kg ha^{-1} (K_1)	7.00e	5.76f
100 kg ha^{-1} (K_2)	7.17e	6.23ef
150 kg ha^{-1} (K_3)	8.47de	7.24def
Removal of first fruiting branch (F_2)		
50 kg ha^{-1} (K_1)	8.64de	8.12cd
100 kg ha^{-1} (K_2)	8.94cd	7.88cd
150 kg ha^{-1} (K_3)	10.53bc	9.36bc
Removal of first and second fruiting branch (F_3)		
50 kg ha^{-1} (K_1)	9.89bcd	8.42bcd
100 kg ha^{-1} (K_2)	9.77bcd	8.71bcd
150 kg ha^{-1} (K_3)	14.19a	13.31a
Removal of all squares from first fruiting branch (F_4)		
50 kg ha^{-1} (K_1)	7.00e	5.82f
100 kg ha^{-1} (K_2)	8.94cd	7.82cde
150 kg ha^{-1} (K_3)	10.66b	9.83b
Removal of all squares from first and second fruiting branch (F_5)		
50 kg ha^{-1} (K_1)	8.88cd	7.41de
100 kg ha^{-1} (K_2)	9.42bcd	8.41bcd
150 kg ha^{-1} (K_3)	14.54a	13.66a
LSD 5 %	**1.656**	**1.589**
Year mean	9.60a	8.53b
LSD 5 %	**0.404**	

Means not sharing a letter in common differ significantly at 5% probability level.

NS= Non-significant,

Table 4.23: Interactive effect of K levels and removal of square/fruiting branch on Cry1Ac concentration (µg g^{-1}) in pericarp of cotton boll

	2011	2012
No fruiting branch removal (F_1)		
50 kg ha^{-1} (K_1)	0.73ef	0.62g
100 kg ha^{-1} (K_2)	0.77def	0.75fg
150 kg ha^{-1} (K_3)	0.89cdef	1.29e
Removal of first fruiting branch (F_2)		
50 kg ha^{-1} (K_1)	0.89cdef	0.92f
100 kg ha^{-1} (K_2)	0.95bcde	1.23e
150 kg ha^{-1} (K_3)	1.10bc	2.00ab
Removal of first and second fruiting branch (F_3)		
50 kg ha^{-1} (K_1)	0.98bcd	1.56d
100 kg ha^{-1} (K_2)	1.03bc	1.89bc
150 kg ha^{-1} (K_3)	1.50a	2.01ab
Removal of all squares from first fruiting branch (F_4)		
50 kg ha^{-1} (K_1)	0.71f	1.20e
100 kg ha^{-1} (K_2)	0.95bcde	1.27e
150 kg ha^{-1} (K_3)	1.15b	1.42de
Removal of all squares from first and second fruiting branch (F_5)		
50 kg ha^{-1} (K_1)	0.92cdef	1.64cd
100 kg ha^{-1} (K_2)	1.00bc	1.81bc
150 kg ha^{-1} (K_3)	1.55a	2.16a
LSD 5 %	**0.299**	

Means not sharing a letter in common within a column differ significantly at 5% probability level.

NS= Non-significant,

branch removal) wherein again increasing potassium supply increased leaf potassium. Overall, control (no fruiting branch removal) remained at the bottom in accumulating potash in cotton leaf. Trend was same during both years of study (table 4.22). Linear regression coefficient (R^2) for potassium concentration (mg g^{-1}) in cotton leaf vs. seed cotton yield per plant (g) was strong and positive as shown in figure 4.24.

Year effect was significant on Cry1Ac concentration (µg/g) in pericarp of cotton boll. During 2011 removal of all squares from first and second fruiting branch and removal of first and second fruiting branch when supplied with higher potassium dose (150 kg ha^{-1}) resulted in more Cry1Ac concentration, while less Cry1Ac concentration in 20 days old boll pericarp was recorded in control (no fruiting branch removal). In 2012, trend was almost similar however, F_2 (removal of first fruiting branch) performed equally well as F_5 (removal of all squares from first and second fruiting branch) and F_3 (removal of first and second fruiting branch) at higher K rate. Coefficient of determination (R^2) of nitrogen concentration (%) and potassium concentration (mg/g) in leaf cotton with Cry1Ac concentration (µg/g) in pericarp of cotton boll was positive (Fig. 4.25).

4.1.10. Discussion

Symptoms of premature senescence spread in cotton crop as season progresses and defoliation occurs; this potassium related premature senescence was found in China (Zheng and Dai, 2000), USA (Oosterhuis, 2001) and Australia (Mott, 2003). In Bt cotton premature senescence symptoms appeared in different experimental units; these symptoms were reddening and browning of top leaves, these are the visible symptoms we could not quantify; for its quantification we took subtended leaf samples from top of plant for determination of nitrogen and potassium concentration. Sufficient potassium is also needed for the efficient use of nitrogen (Varco and Fridgen, 2004). Previous findings showed that two early basal fruiting branches removal had non-significant effect on K concentration (Dong *et al*., 2008) but their study was on two basal fruiting branches removal with no K application; in our study increased N concentration in cotton leaf in may be due to potassium application that enhanced N uptake in plant. Increased concentration of K in cotton leaf in K_3 was due to higher application rate of potassium. Result of an experiment showed that leaf Cry1Ac protein expression (four days prior to crop defoliation) in late growing season was not affected by applied potassium. Insect pest observations indicated that Cry1Ac protein expression was sufficient to control Heliothine larvae

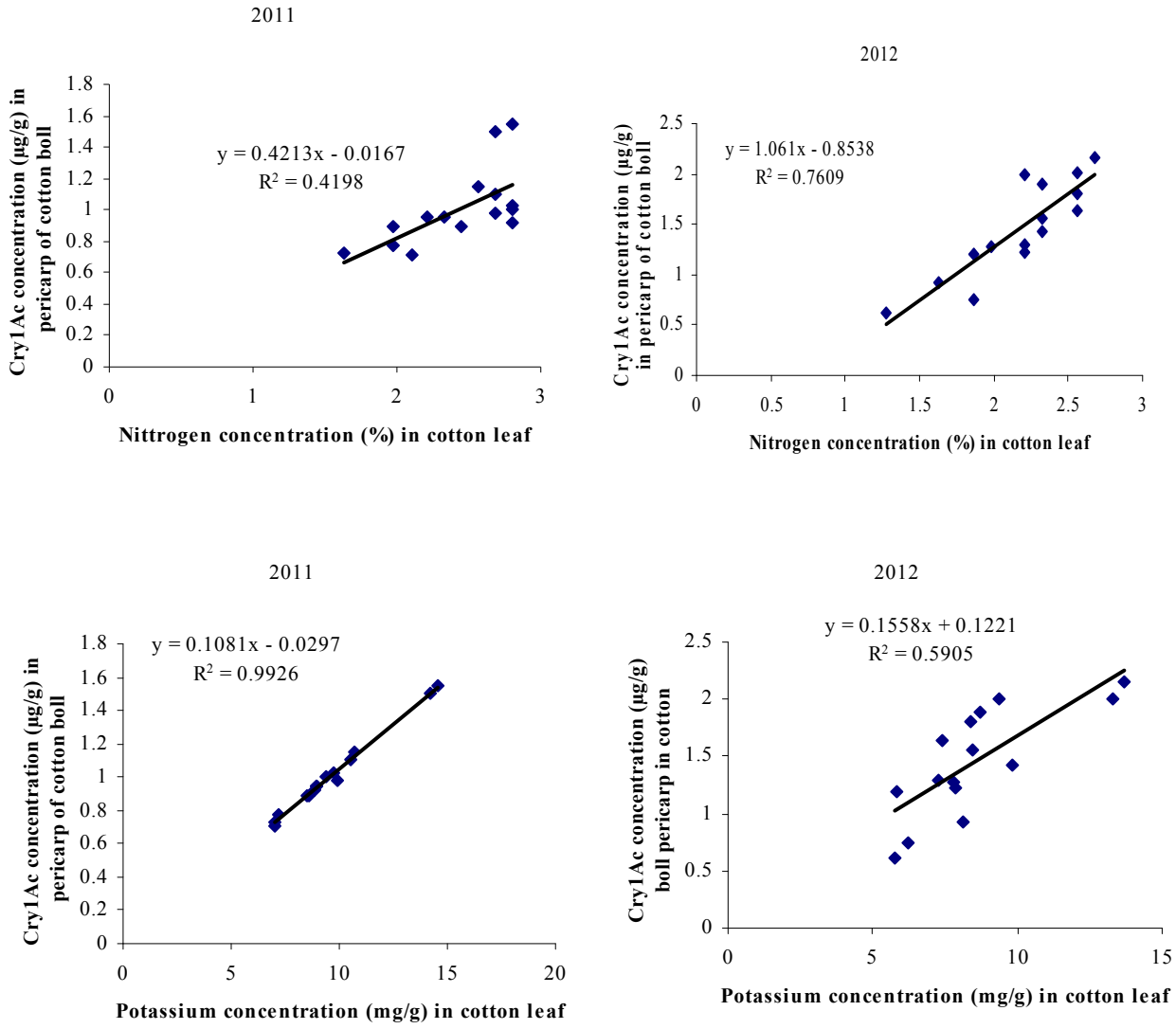


Fig. 4.25: Relationship of nitrogen concentration (%) and potassium concentration (mg/g) in leaf cotton with Cry1Ac concentration (µg g^{-1}) in pericarp of cotton boll

in the experiment (Rochester, 2006). Introduced genes (Bt and CpTI) that encode for insecticidal protein and their introduction processes surely affected tolerance to low potassium in Bt cotton (Li *et al*., 2008). In this study there was more Cry1Ac concentration in treatment where two fruiting branches were removed or where all squares were removed from 1st and 2nd fruiting branches with higher potassium application; this may be due to more sources and less fruit at initial stage leading to good balance between source/sink at later stage and also due to higher N and K in plants that might have helped in early healing up of the injury caused by square/branch removal.

4.1.11. Economic analysis

Net field benefit (NFB), benefit cost ratio (BCR) and marginal rate of return (MRR) were calculated on the basis of data of 2011 and 2012. In 2011 Bt cotton grown with square removal from first and second fruiting branches at higher level of potassium application (150 kg ha^{-1}) gave maximum NFB of Rs. 185467 ha^{-1} followed by F_3 (removal of first and second fruiting branch) at higher K dose with NFB of Rs. 181683 ha^{-1} (table 4.24). While maximum BCR (2.79 & 2.92) and MRR (812 & 944) were recorded with removal of first and second fruiting branch with higher level (150 kg ha^{-1}) of potassium application, during both study years (table 4.24 and 4.25).

Table 4.24: Interactive effect of K levels and removal of square/fruiting branch in cotton on net income (Rs ha^{-1}), benefit cost ratio (BCR) and marginal rate of return (MRR%) during 2011

	Costs that vary	Marginal Costs	Total cost	Gross income	Net income	Marginal net income	BCR	MRR
No fruiting branch removal (F_1)								
50 kg ha^{-1} (K_1)	5000		90176	186854	96678		2.07	
100 kg ha^{-1} (K_2)	10000	5000	95176	192857	97680	1002	2.02	20
150 kg ha^{-1} (K_3)	15000	5000	100176	193555	93378	-4302	1.93	-86
Removal of first fruiting branch (F_2)								
50 kg ha^{-1} (K_1)	6070		91247	201303	110056		2.20	
100 kg ha^{-1} (K_2)	11070	5000	96247	217357	121110	11054	2.25	221
150 kg ha^{-1} (K_3)	16070	5000	101247	240670	139423	18313	2.37	366
Removal of first and second fruiting branch (F_3)								
50 kg ha^{-1} (K_1)	6249		91425	219870	128444		2.40	
100 kg ha^{-1} (K_2)	11249	5000	96425	237459	141034	12589	2.46	251
150 kg ha^{-1} (K_3)	16249	5000	101425	283108	181683	40649	2.79	812
Removal of all squares from first fruiting branch (F_4)								
50 kg ha^{-1} (K_1)	7498		92674	220986	128312		2.38	
100 kg ha^{-1} (K_2)	12498	5000	97674	215682	118007	10304	2.20	-206
150 kg ha^{-1} (K_3)	17498	5000	102674	254211	151537	33529	2.47	670
Removal of all squares from first and second fruiting branch (F_5)								
50 kg ha^{-1} (K_1)	8747		93923	213588	119664		2.27	
100 kg ha^{-1} (K_2)	13747	5000	98923	254141	155218	35553	2.56	711
150 kg ha^{-1} (K_3)	18747	5000	103923	289390	185467	30249	2.78	604

Table 4.25: Interactive effect of K levels and removal of square/fruiting branch in cotton on net income (Rs ha^{-1}), benefit cost ratio (BCR) and marginal rate of return (MRR%) during 2012

	Costs that vary	Marginal Costs	Total cost	Gross income	Net income	Marginal Net income	BCR	MRR
No fruiting branch removal (F_1)								
50 kg ha^{-1} (K_1)	5000		90176	196766	106589		2.18	
100 kg ha^{-1} (K_2)	10000	5000	95176	209679	114502	7913	2.20	158
150 kg ha^{-1} (K_3)	15000	5000	100176	213797	113620	-881	2.13	-17
Removal of first fruiting branch (F_2)								
50 kg ha^{-1} (K_1)	6070		91247	220847	129600		2.42	
100 kg ha^{-1} (K_2)	11070	5000	96247	229362	133115	3515	2.38	70
150 kg ha^{-1} (K_3)	16070	5000	101247	262099	160851	27736	2.58	554
Removal of first and second fruiting branch (F_3)								
50 kg ha^{-1} (K_1)	6249		91425	237459	146034		2.59	
100 kg ha^{-1} (K_2)	11249	5000	96425	244160	147734	1700	2.53	34
150 kg ha^{-1} (K_3)	16249	5000	101425	296370	194945	47210	2.92	944
Removal of all squares from first fruiting branch (F_4)								
50 kg ha^{-1} (K_1)	7498		92674	225663	132988		2.43	
100 kg ha^{-1} (K_2)	12498	5000	97674	230060	132386	-602	2.35	-12
150 kg ha^{-1} (K_3)	17498	5000	102674	270195	167521	35135	2.63	702
Removal of all squares from first and second fruiting branch (F_5)								
50 kg ha^{-1} (K_1)	8747		93923	231945	138021		2.46	
100 kg ha^{-1} (K_2)	13747	5000	98923	257562	158638	20616	2.60	412
150 kg ha^{-1} (K_3)	18747	5000	103923	302094	198170	39532	2.90	790

The present investigations were carried out to find out interactive effect of square/fruiting branch removal using different nitrogen and potassium doses. Effects of changes in cotton architecture were studied at the Agronomic Research Area, University of Agriculture Faisalabad. Two experiments were planted during 2011 and repeated during 2012. The summary result of each experiment is given below.

Experiment: I

Study comprised of manual alteration of plant architecture i.e. F_1: no fruiting branch removal, F_2: removal of first fruiting branch, F_3: removal of first and second fruiting branch, F_4: removal of all squares (floral bud) from first fruiting branch, F_5: removal of all squares from first and second fruiting branch; and nitrogen rates i.e. N_1: 175, N_2: 225 and N_3: 275 kg ha^{-1}. Delayed flowering and first boll spilition were recorded in removal of first and second fruiting branch (F_3) against the earliest flowering and first boll spilition in F_1 (no fruiting branch removal). More nodes for first fruiting branch and taller first fruiting branch were recorded in F_5 (removal of all squares from first and second fruiting branch) closely followed by F_4 (removal of all squares from fruiting branch) and F_3 (removal of first and second fruiting branch). Among the nitrogen levels more number of days to first flower, days to first boll opening, node number for first fruiting branch and first fruiting branch height were recorded in higher level of nitrogen dose (275 kg ha^{-1}) followed by medium (225 kg N ha^{-1}) and lower (175 kg ha^{-1}) nitrogen application. Less boll maturation period was recorded in F_4 (removal of all square from first fruiting branch) and F_5 (removal of all squares from first and second fruiting branch) while F_1, F_2 and F_3 have more boll maturation period. Lower earliness indices were observed in F_5 and F_3 against the minimum in F_1. Boll maturation period, earliness index and seed index were more with 275 kg N ha^{-1}. More nodes above white flower (NAWF) and nodes above cracked boll (NACB) were recorded in removal of all squares from first and second fruiting branch and removal of these two branches followed by removal of all squares from first fruiting branch or removal of this branch and least values for these characters were recorded in control. Among nitrogen levels maximum NAWF & NACB were recorded with higher nitrogen application followed by medium and low

nitrogen dose during both years of study. More average boll weight per plant, more number of insects' damaged bolls per plant and less number of unopened bolls per plant were recorded with higher nitrogen application. More number of opened bolls per plant and seed cotton yield per plant was recorded in F_5, closely followed by F_3 and F_4 with higher level of nitrogen application while minimum was recorded in F_1 regardless of nitrogen dose. More monopodial and sympodial branches per plant were recorded in removal of all squares from first and second fruiting branch and removal of first and second fruiting branch with higher and medium level of nitrogen application while minimum was recorded in control. Increasing nitrogen application increased total bolls per plant and cotton yield to highest level in removal of first and second fruiting branch and removal of all squares from first and second fruiting branch than other treatments. Before manual alteration of the plants architecture, no variation in plant height was observed at squaring stage, but at physiologically cut-out stage and at last pick, plants gained more height with removal of all squares from 1st and 2nd fruiting branch and removal of first and second fruiting branch with maximum level of nitrogen application and less plant height was recorded with no fruiting and/or square removal with minimum nitrogen application. Ginning out turn, fiber length, seed oil and seed protein content were influenced by fruiting branch or square removal but difference was less. Increasing nitrogen improved seed and fiber quality. Highest nitrogen concentration in cotton leaf was observed in F_5 and minimum was recorded in F_1, similarly leaf nitrogen concentration increased with increasing nitrogen rates. Potassium concentration in leaf increased with increasing nitrogen application in F_3 and F_5 treatment while in F_1, F_2 and F_3 medium and higher nitrogen application was statistically at par with each other. More Cry1Ac concentration in 20 days old bolls was recorded in F_5 and F_3 with higher level of nitrogen application and less Cry1Ac concentration was recorded in F_1 at lower nitrogen application. Finally effectiveness of the studied treatments was checked by economic and marginal analysis. In 2011 Bt cotton grown with square removal from first and second fruiting branches at higher level of nitrogen application (275 kg ha^{-1}) gave maximum NFB of Rs. 168105 ha^{-1} followed by F_3 (removal of first and second fruiting branch) at higher N dose with NFB of Rs. 159156 ha^{-1}; while minimum NFB of Rs. 87353 ha^{-1} was associated with F_2 (removal of first fruiting branch) at low level of nitrogen application; almost similar trend was seen during 2012. Maximum BCR (2.65) was recorded with removal of all squares from first and second fruiting branch at higher level of nitrogen application followed by 2.60 (BCR) in removal of first and

second fruiting branch at higher level of nitrogen application and minimum BCR (1.92) was recorded in control (no fruiting branch removal) at medium level of nitrogen application (225 kg ha^{-1}) during 2011 while in 2012 maximum BCR (3.00) was recorded in F_3 (removal of first and second fruiting branch) at higher nitrogen dose. While maximum MRR (1839 & 2115) was recorded with removal of first and second fruiting branch with medium level (225 kg ha^{-1}) of nitrogen application, during both study years.

Experiment: II

Experiment comprised of manual alteration of plant architecture i.e. no fruiting branch removal (F_1), removal of first fruiting branch (F_2), removal of first and second fruiting branch (F_3), removal of all squares (floral bud) from first fruiting branch (F_4), removal of all squares from first and second fruiting branch (F_5); and potassium rates i.e. K_1:50, K_2: 100 and K_3: 150 kg K ha^{-1}. Days to squaring remained non-significant by square/fruiting branch removal. More days were taken to first flowering and first boll spilition in removal of first and second fruiting branch and less days taken to first flowering and first boll spilition was recorded in control (no fruiting branch removal). Less boll maturation period was recorded in removal of all squares from first and second fruiting branch (F_5) and removal of all square from first fruiting branch (F_4) while control (F_1), removal of first fruiting branch (F_2) and removal of first and second fruiting branch (F_3) had more boll maturation period. More nodes for first fruiting branch and taller first fruiting branches were recorded in F_5 (removal of all squares from first and second fruiting branch) which was at-par with F_3 (removal of first and second fruiting branch) and less nodes for first fruiting branch and minimum first fruiting branch height were observed in F_1 (no fruiting branch removal). Among the potassium levels more number of days to first flowering, boll opening, node numbers for first fruiting branch and first fruiting branch height were recorded with higher potassium dose (150 kg ha^{-1}) while medium (100 kg K ha^{-1}) and lower (50 kg K ha^{-1}) potassium levels were at-par with each other. Lower earliness index was observed in F_5 and F_3 and higher earliness index was observed in F_1. More boll maturation period, earliness index and seed index were recorded with the application of 150 kg K ha^{-1} while medium and low potassium levels were at-par with each other. More nodes above white flower (NAWF) and nodes above cracked boll (NACB) were recorded in F_5, followed by F_3, F_4 and F_2 while minimum values for these parameters were recorded in control (F_1). Among potassium levels maximum NAWF & NACB

were recorded with higher potassium application followed by medium and low potassium dose during both years of study. More average boll weight per plant, rotted bolls, number of insects' damaged bolls per plant and less number of unopened bolls per plant were recorded with higher potassium application while less average boll weight per plant, less number of insects' damaged bolls per plant and more number of unopened bolls per plant was recorded with lower dose of potassium application however, fruiting branch and/or square removal has no effect on these parameters. More number of opened bolls per plant and seed cotton yield (per plant and per hectare) were recorded in F_5, followed by F_3 and F_4 with higher level of potassium application while minimum was recorded in control (F_1) at all levels of potassium application. More monopodial and sympodial branches per plant were recorded in removal of all squares from first and second fruiting branch and removal of first and second fruiting branch with higher level of potassium application while less was recorded in control at all levels of potassium. No variation in plant height was observed at squaring stage, but at physiologically cut-out stage plants gained more height with removal of all squares from 1st and 2nd fruiting branch and removal of first and second fruiting branch with maximum level of potassium application and less plant height was recorded with no fruiting and/or square removal with minimum potassium application; similar trend was recorded for plant height at last pick. Ginning out turn, fiber length, seed oil and seed protein content were influenced by fruiting branch or square removal but difference was less. Increasing potassium improved seed and fiber quality. More nitrogen concentration in cotton leaf was observed in removal of all squares from first and second fruiting branch (F_5) and removal of first and second fruiting branch (F_3) while minimum was recorded in control (F_1), similarly leaf nitrogen concentration increased with increasing potassium dose. Potassium concentration in leaf increased with increasing potassium application in F_3, F_5 and F_4 treatment while in F_1, F_2 medium and higher potassium levels were statistically at par with each other. More Cry1Ac concentration in 20 days old bolls was recorded in F_5 and F_3 with higher potassium dose and less Cry1Ac concentration was recorded in control at lower potassium application. Finally economic analysis of the studied treatments was carried out. In 2011 Bt cotton grown with square removal from first and second fruiting branches at higher level of potassium application (150 kg ha^{-1}) gave maximum NFB of Rs. 185467 ha^{-1} followed by F_3 (removal of first and second fruiting branch) at higher K dose with NFB of Rs. 181683 ha^{-1}; while minimum NFB of Rs. 96678 ha^{-1} was associated with F_1 (no fruiting branch removal) at low level of potassium application; almost

similar trend was seen during 2012. Maximum BCR (2.79) was recorded with removal of first and second fruiting branch at higher level of potassium application followed by 2.78 (BCR) in removal of all squares from first and second fruiting branch at higher level of potassium application during 2011; second study year followed similar trend. While maximum MRR (812 & 944) was recorded with removal of first and second fruiting branch with higher level (150 kg ha^{-1}) of potassium application, during both study years.

CONCLUSION

Removal of first and second fruiting branch and removal of all squares from first and second fruiting branch along with increasing nitrogen dose helped in delayed onset of senescence in cotton as well as in increasing Cry1Ac concentration in boll pericarp. Although maximum net income and BCR were recorded with removal of all squares from first and second fruiting branch using 275 kg N ha^{-1} (F_5N_3) but on the basis of MRR we conclude that removal of first and second fruiting branch with 225 kg N ha^{-1} (F_3N_2) is the most economical treatment combination. Senescence was also delayed with each increase in K application. Removal of first and second fruiting branch (F_3) or removal of all squares from these branches (F_5) with 150 kg K ha^{-1} increased the Cry1Ac concentration to the highest in boll pericarp. Highest BCR as well as maximum MRR were obtained by supplying the crop with 150 kg K ha^{-1} and removing first and second fruiting branches at early stages.

SUGGESTION FOR FUTURE RESEARCH

Future studies should focus on removal of fruiting branches instead of square removal; although more or less both performed equally well but former is easier and quicker and Agricultural Engineers can try it to manage mechanically. Response of cotton plant to fruiting branch removal should be studied while readjusting its R x R and P x P distances. Fruiting branch removal studies must be carried out while using different nutrients combinations to look into the behaviour of Cry1Ac (Bt-toxin) expression. Cry1Ac expression must also be studied in relation to different climatic factors.

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Fixed cost of production of experiment I during 2011 and 2012

S. No.	Description	No.	Cost (Rs.) unit^{-1}	Total cost (Rs.)
	Input cost			
1	Pre sowing			
	Cultivation	2	1500	3000
	Planking	2	1000	2000
	Ride making	1	1500	1500
2	Seed and sowing			
	Seed cost	15 kg	150	2250
	Labour charges	4 men/ha	350	1400
	Thinning	2 men/ha	350	700
3	Irrigation			
	Cleaning of water course	4 men/ha	350	1400
	Labour charges for 9 irrigation	1 men/ha	350	3150
	Tube well irrigation	9	1000	9000
4	Intercultural operations			
	Herbicide			
	Per emergence	1	800	800
	Post emergence	1	400	400
	Spray application charges	2 men/ha	350	700
5	Plant protection			
	Insecticides		2190	2190
	Spray application charges	5 men/ha	350	1750
6	Fertilizer			
	87:100 P: K kg ha^{-1}		18197	18197
7	**Land rent**		35000	35000
8	Picking charges	per kg	5	
9	Support price recommended	40 kg	2825	
10	Fixed cost			83437

Fixed cost of production of experiment II during 2011 and 2012

S. No.	Description	No.	Cost (Rs.) unit^{-1}	Total cost (Rs.)
	Input cost			
1	Pre sowing			
	Cultivation	2	1500	3000
	Planking	2	1000	2000
	Ridge making	1	1500	1500
2	Seed and sowing			
	Seed cost	15 kg	150	2250
	Labour charges	4 men/ha	350	1400
	Thinning	2 men/ha	350	700
3	Irrigation			
	Cleaning of water course	4 men/ha	350	1400
	Labour charges for 9 irrigation	1 men/ha	350	3150
	Tube well irrigation	9	1000	9000
4	Intercultural operations			
	Herbicide			
	Per emergence	1	800	800
	Post emergence	1	400	400
	Spray application charges	2 men/ha	350	700
5	Plant protection			
	Insecticides		2190	2190
	Spray application charges	5 men/ha	350	1750
6	Fertilizer			
	87:100 P: K kg ha^{-1}		19936	19936
7	Land rent		35000	35000
8	Picking charges	per kg	5	
9	Support price recommended	40 kg	2825	
10	Fixed cost			85176

Statement Concerning Data

The replicated raw data, detailed analysis of variance and regression analysis have been lodged in the department of Agronomy, University of Agriculture Faisalabad, Pakistan. Any person interested may approach to Dr. Muhammad Farrukh Saleem, Incharge Analytical Laboratory, Department of Agronomy, University of Agriculture Faisalabad, Pakistan, for use of data.