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**Full Length Article**

# Evaluating the Response of Nitrogen Application on Growth, Development and Yield of Quinoa Genotypes

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## Abstract

Quinoa *(Chenopodium quinoa* Willd.), a pseudocereal, is a new introduction in Pakistan. Crop is abiotic stress tolerant, low input requiring and economic produce is grain of superior nutritional profile as compared to common cereals. Its production technology has yet to be explored according to local conditions. For obtaining high crop yields nutrient in balanced amount is a basic requirement. To evaluate the influence of different nitrogen doses on the productivity of quinoa, a field experiment was conducted on two promising genotypes A9 and CPJ-2 with five nitrogen levels (0, 50, 75, 100, 125 kg $ha^{-1}$) in two splits. Nitrogen fertilization (75 kg N $ha^{-1}$ and higher N levels) caused early completion of vegetative phenological stages. Genotype CPJ2 was early maturing as compared to genotype A9. Soil application of nitrogen (75 kg N $ha^{-1}$ and higher N levels) improved all growth and yield related traits resulting in increased biomass production, economic harvest and harvest index. Percent increase in grain yield over control was also affected by nitrogen application. Comparatively genotype A9 was high yielding than CPJ-2. Furthermore, N supplementation of soil (75 kg N $ha^{-1}$ and higher N levels) improved leaf chlorophyll content of both quinoa genotypes. In conclusion, soil application with N @ 75 kg N $ha^{-1}$ was found best level of N to attain maximum economic harvest of quinoa. 

**Keywords:** Pseudocereal; Nitrogen application; Yield

## Introduction

On an average four major crops of Pakistan i.e. wheat, rice, cotton, and sugarcane contribute about 28% to the value added in overall agriculture and 5.9% to GDP of the country (Govt. of Pakistan, 2011). Dependence on these few choices leads to food devastation at national level. The situation is further aggravated by the limitations of crop production i.e. unavailability of quality seed and fertilizers, abiotic stresses like freezing, chilling, frost, heat and problem soils.

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal, native to Latin America which has the potential to grow with less inputs, water and tolerate a variety of biotic and abiotic stresses (Rea *et al*., 1979), which if introduced can be a good substitute to existing cereals, able to sustain on a variety of problem soils and withstand a range of abiotic stresses with highly nutritive importance. Preliminary success of quinoa cultivation in Pakistan has opened the avenues to explore the production potential of quinoa comprehensively (Munir *et al*., 2009). Quinoa can be successfully grown on marginal soils showing its very low nutrient requirements (Jacobsen, 2003). However, quinoa is also highly responsive to soil nitrogen (Erley *et al*., 2005).

More production improvements of pseudocereals are needed. It has not been explained yet that to which degree this may be attained by an enhanced N fertilization. Number of investigations on fertilization of N needed by pseudocereals, are very less. Yield of Amaranth, another pseudocereal was improved in many environments by N fertilization (Elbehri *et al.*, 1993), however, grain yield was not increased with excessive levels of N fertilization (90 kg N $ha^{-1}$) (Myers, 1998). Erley *et al.* (2005) reported quinoa responded well to N and achieved yield up to 3.5 Mg $ha^{-1}$ at 120 kg N $ha^{-1}$, grain yield boosted by 94% as compared to control. However, Jacobsen *et al.* (1994) found increase in yield (averaging 12%) at 80 to 120 kg N $ha^{-1}$. Quinoa responds to N application by not only increase the crop growth and yield but also the quality of grain. Thanapornpoonpong *et al.* (2008) explored the effect of different nitrogen rates (0.16 and 0.24 g N $kg^{-1}$ soil) on protein content of seed and amino acid profile of amaranth and quinoa. Nitrogen fertilization effected amino acid content of quinoa and amaranth. Both had rich lysine contents (6.3-8.2 g 100 $g^{-1}$ protein) but low methionine (1.28 g 100 $g^{-1}$protein). Thus, diets of humans can be improved by maintaining and increasing essential amino acid content and proteins by applying N fertilizer.

So, the investigation was carried out to determine the optimum level of nitrogen for getting maximum yield of quinoa genotypes under agro-ecological condition of Faisalabad, Pakistan.

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## Materials and Methods

Seeds of promising quinoa genotypes CPJ2 and A9 were University of Agriculture, Faisalabad, Pakistan. The crop wcollected from the Department of Crop Physiology, Uas sown on ridges during December, 2010 in 75 cm spaced rows maintaining 15 cm plant to plant distance using seed rate of 18 kg ha$^{-1}$. DAP fertilizer were applied @ 65 kg P ha$^{-1}$. Five nitrogen levels (0, 50, 75, 100, 125 kg ha$^{-1}$) were applied in two splits. Whole of the phosphorus and 1/2 of nitrogen doses were applied as basal dose and the remaining were applied at flowering stage. Genotypes CPJ2 and A9 were harvested at harvest maturity on 15$^{th}$ and 28$^{th}$ of April, 2011, respectively. The soil texture was sandy loam with pH 8 and NPK levels were 0.042%, 11.52 mg/kg, 90 mg/kg respectively.

Phenological data regarding time taken to emergence, true leaves stage, multiple leaves stage, bud formation panicle emergence stage, flowering stage, milk stage and maturity were determined by regular crop visits. Crop growth rate and leaf area index (LAI) were calculated by using the formula given by Hunt (1978). Leaf area was measured with digital leaf area meter (Model CI-203 (CID Bioscience, USA). Leaf area index (LAI) as calculated above was used for the determination of leaf area duration (LAD) for the entire growing season (Hunt, 1978).

Stem diameter, plant height and number of panicles were measured manually from five randomly selected plants. After harvesting, crop was tied into bundles and sundried for a week. The crop was threshed manually and yield related traits i.e., grain weight, 1000-grains were determined.

Plant leave samples were taken at panicle emergence stage from each plot and were kept in biomedical freezer at -30°C. Chlorophyll contents in plant leaves were determined by the method described by the Bruinsma (1963).

## Results

### Phonological Development

Similar trend was observed for both quinoa genotypes to attain true leaves and multiple leaf stages at all N levels (Table 1). Both genotypes took less time to attain true leaves stage and multiple leaf stage at 75 and 100 kg N ha$^{-1}$ respectively. Bud formation and panicle emergence were completed earlier in genotype CPJ2 than genotype A9 and time reduced with the increasing N level. Similarly, CPJ2 plants completed flowering and milking stages earlier as compared to A9. Genotype CPJ-2 attained early maturity in all plots of N fertilization, while high rate N delayed maturity in genotype A9. Delayed maturity was observed in genotype A9 at highest rate of N and earliest maturity in control plots.

### Growth and Development

Crop growth rate (CGR) of both genotypes was almost similar at all nitrogen levels before panicle emergence. However, the trend was significant different among both genotypes after panicle emergence (Fig. 1). Maximum CGR was observed in both genotypes in soil applied with 75 kg N ha$^{-1}$ before panicle emergence, whereas after panicle emergence the response of A9 was comparatively better. Moreover, CGR of both genotypes was also improved with increasing levels of nitrogen (100 and 135 kg N ha$^{-1}$). Regarding leaf area index (LAI), both genotypes responded differently at 40 and 55 d after emergence. At 70 d after emergence, both genotypes responded in similar way and maximum LAI was observed in A9 at 75 kg N ha$^{-1}$. An increasing trend was recorded in both genotypes with increasing application of N level (Fig. 2). N also affected seasonal leaf area duration (SLAD) of quinoa crop before and after panicle emergence stages (Fig. 3). The response of A9 was better than CPJ2. Maximum SLAD (27.09 d) before panicle emergence was attained by genotype A9 at 75 kg N ha$^{-1}$ which was statistically similar to the values obtained at higher levels (100 and 125 kg N ha$^{-1}$) for the same genotype.

### Yield Related Traits

N significantly improved yield and yield related attributes (Table 2). Except number of panicles per plant, all yield related attributes remained non-significant. Panicle length was maximally enhanced in both quinoa genotypes at 100 kg N ha$^{-1,}$ which was statistically at par to higher nitrogen levels (75 and 125 kg N ha$^{-1}$). Comparatively, A9 showed better response with or without nitrogen application. At 75 kg N ha$^{-1}$, highest number of panicle per plant was recorded

**Table 1:** Influence of nitrogen application on phenological development of two quinoa genotypes

| Nitrogen levels (kg ha$^{-1}$) | Days to true leaf stage | | Days to multiple leave | | Days to bud formation stage | | Days to panicle emergence | | Days to flowering | | Days to milk stage | | Days to maturity | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CPJ2 | A9 | CPJ2 | A9 | CPJ2 | A9 | CPJ2 | A9 | CPJ2 | A9 | CPJ2 | A9 | CPJ2 | A9 |
| 0 | 3.66 a | 3.66 a | 17.66 ab | 18.33 a | 27.33 bc | 31.00 a | 38.33 cd | 43.00 a | 51.66 b | 58.00 a | 101.33 c | 114.00 a | 111.67 d | 127.00 c |
| 50 | 3.33 ab | 3.66 a | 17.33 abc | 17.66 abc | 27.00 c | 29.00 b | 37.33 d | 41.33 ab | 51.33 b | 58.00 a | 101.33 c | 112.67 ab | 112.00 d | 128.67 bc |
| 75 | 2.66 b | 2.66 b | 15.33 cd | 16.66 a-d | 24.00 d | 25.66 cd | 34.00 e | 39.33 c | 52.00 b | 59.00 a | 100.33 c | 112.00 b | 112.00 d | 130.33 ab |
| 100 | 3.00 ab | 3.00 ab | 15.00 d | 16.33 bcd | 24.33 d | 27.00 c | 34.33 e | 40.00 bc | 51.00 b | 60.00 a | 101.00 c | 112.67 ab | 112.67 d | 131.00 a |
| 125 | 3.33 ab | 3.33 ab | 15.33 cd | 16.66 a-d | 25.66 cd | 27.00 c | 34.67 e | 40.00 bc | 51.66 b | 58.33 a | 100.60 c | 113.30 ab | 113.00 d | 131.33 a |
| P < 0.05 | 0.86 | | 1.69 | | 1.97 | | 1.67 | | 2.28 | | 1.60 | | 1.900 | |

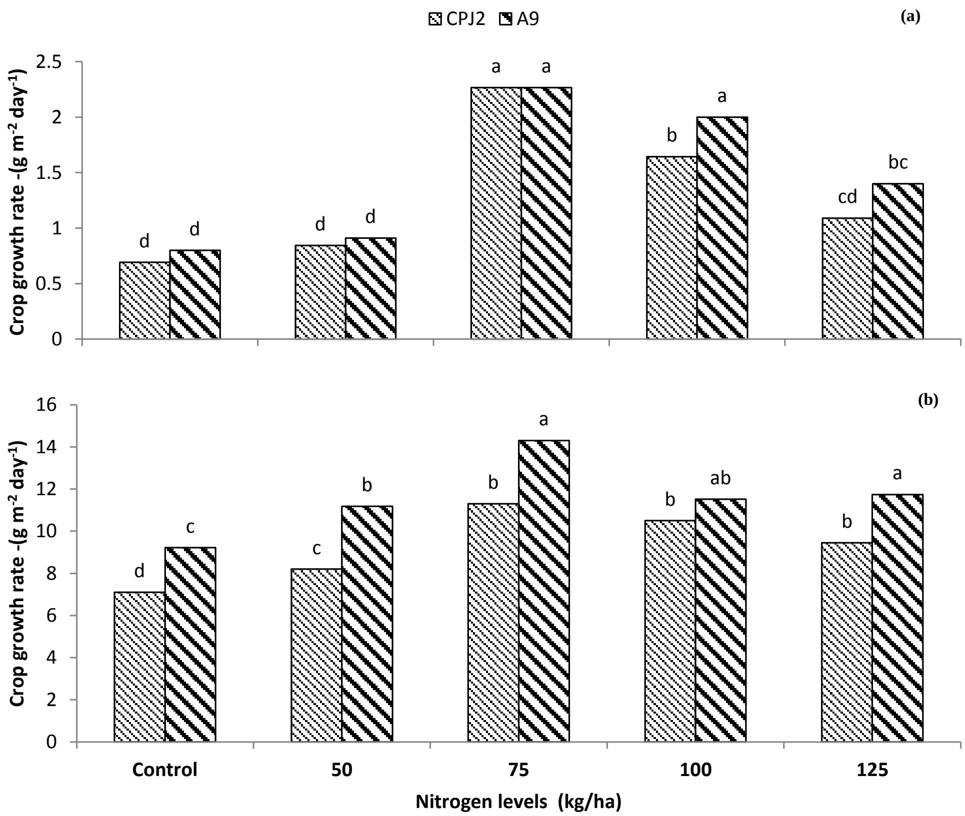


**Fig. 1:** Influence of nitrogen application on crop growth rate (g $m^{-2}$ $day^{-1}$) before (a) and after (b) panicle emergence of two quinoa genotypes

**Table 2:** Influence of nitrogen application on yield and yield related traits of two quinoa genotypes

| Nitrogen levels (kg $ha^{-1}$) | Plant height (cm) | | Stem diameter (cm) | | Main panicle length (cm) | | Number of panicle per plant | | Thousand grain weight (g) | | Biological Yield (kg $ha^{-1}$) | | Economic yield (kg $ha^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CPJ2 | A9 | CPJ2 | A9 | CPJ2 | A9 | CPJ2 | A9 | CPJ2 | A9 | CPJ2 | A9 | CPJ2 | A9 |
| 0 | 70.11 e | 97.89 bc | 0.712 g | 0.872 ef | 20.33 cd | 16.33d | 7.00f | 12.33 d | 2.14 a | 2.12 a | 7.14 e | 9.02 e | 0.82 e | 1.17 de |
| 50 | 82.78 d | 117.00 a | 0.76 fg | 0.93 de | 12.21cd | 20.33 cd | 7.33 ef | 15.66 c | 2.19 a | 2.17 a | 14.17 d | 15.46 cd | 1.68 cd(105)* | 2.08 bc (77) |
| 75 | 97.89 bc | 121.22 a | 1.08 bcd | 1.35 a | 27.66ab | 23.66 bc | 9.66 d | 19.33 a | 2.02 a | 2.00 a | 16.46 bcd | 20.47 ab | 2.02 c (145) | 2.85 a (146) |
| 100 | 87.11 cd | 120.22 a | 1.03 cd | 1.19 b | 29.00a | 25.00 ab | 9.33 def | 19.00 ab | 1.90 a | 1.87 a | 16.30 bcd | 22.59 a | 1.95 c (137) | 3.12 a (166) |
| 125 | 99.00 b | 121.00 a | 0.96 de | 1.18 bc | 27.66 ab | 23.66 bc | 8.66 def | 16.66 bc | 2.11 a | 2.09 a | 14.72 cd | 19.46abc | 1.78 cd (116) | 2.66ab(127) |
| $P < 0.05$ | | | | | 4.65 | | 2.69 | | 0.741 | | 4.72 | | 0.59 | |

Figures sharing different letters in each column are statistically significant at $P < 0.05$; *Values in parenthesis indicates % increase in grain yield over control

in both genotypes. 75 and 125 kg N $ha^{-1}$ application produced more biological and grain yields in both genotypes. Additionally, plant height and stem diameter were significantly improved with the application of nitrogen (Table 2).

### Leaf Chlorophyll Contents

Chlorophyll contents of both quinoa types increased with increase in N level (Fig. 4). Maximum chlorophyll *a* and *b* contents were observed in both genotypes at 75 kg N $ha^{-1}$. Peak value for total chlorophyll content (4.020 and 3.80 mg $g^{-1}$ fresh weight) were recorded in genotypes A9 and CPJ2, respectively at 75 kg N $ha^{-1}$.

## Discussion

Phenology is an important factor, which fixes crop growth period, affects crop production and helps to determine the growth behavior of a crop under a specific climatic conditions. Nitrogen fertilization is reported to effect developmental stages of crop (Thanapornpoonpong *et al.*, 2008; Khan *et al.*, 2013). Present study indicates that the time taken from emergence to true leaves was not significantly affected by N application, which indicates that nitrogen application seemed to be sufficient at the early

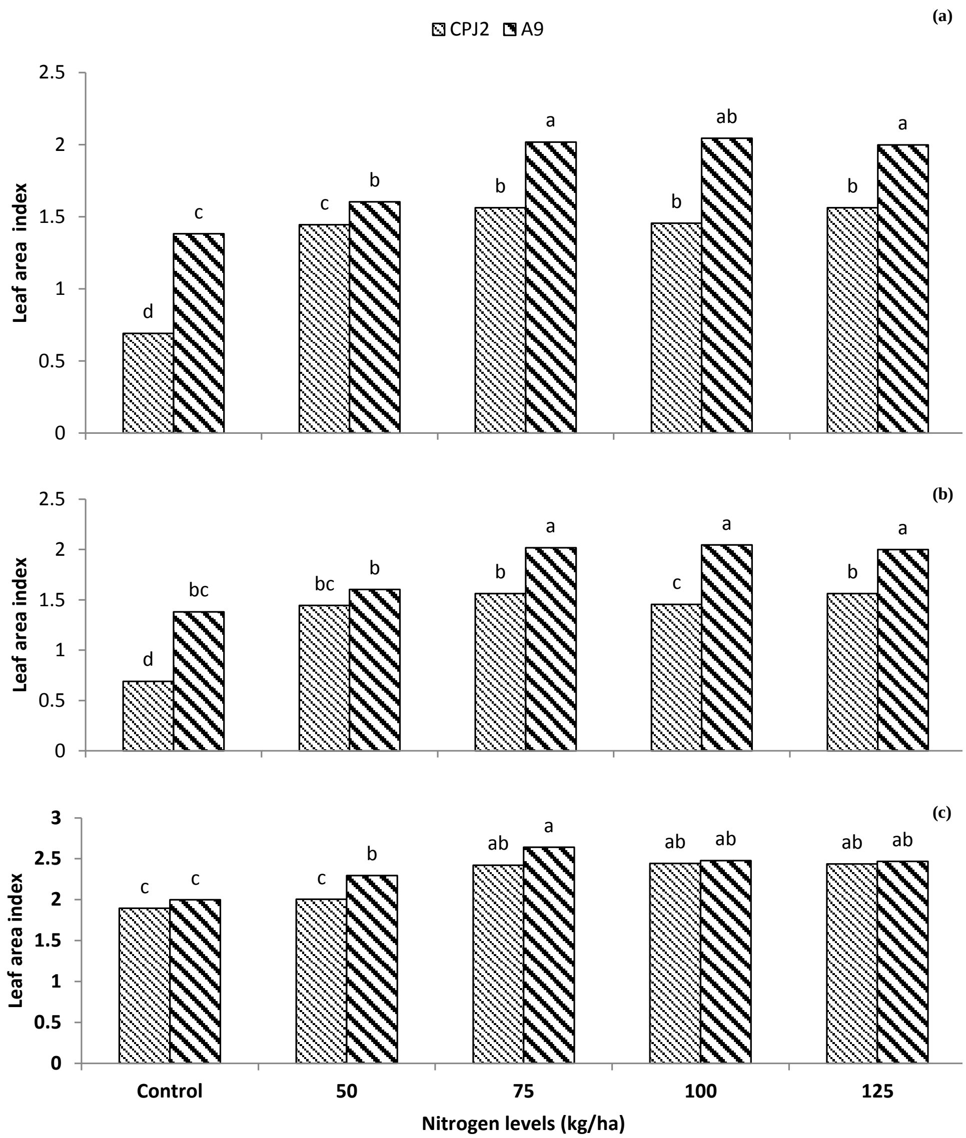


**Fig. 2:** Influence of nitrogen application on leaf area index during 40 (a), 55 (b) and 70 (c) days after emergence of two quinoa genotypes

growth stages and later on nitrogen effects on other developmental stages (Uhart and Andrade, 1995). It might be due to negligible leaf area which has little role in plant growth and development at this growth stage (Amaliotis *et al*., 2004). Nitrogen significantly affected the developmental stage of emergence to four leaves of quinoa genotypes (Table 1). After emergence of photosynthetic active leaves (true leave), four leave stage and multiple leave stage were affected by N supplementation. Leaf emergence was earlier in plots treated with high N levels. Supplementation of rhizosphere with nitrogen also significantly reduced time to start reproductive stage (bud formation) and time to panicle emergence of quinoa crop. These phenological events were completed earlier in genotype CPJ2 when N dose of 75 kg $ha^{-1}$ was applied while genotype A9 was late to show these phenological responses in control plots. The possible reasons may be the difference in phenology behavior between genotypes regarding early or late maturing nature.

The results of current study also indicate that time to complete flowering and days to milk stage was not affected by N. However, quinoa genotypes showed different response relating this developmental stage. CPJ2 showed earlier response as compared to genotype A9. It might be due to high temperature (mid-March) of growing area which resulted in uniform achievement of these developmental stages. Extreme temperatures have a notable effect on

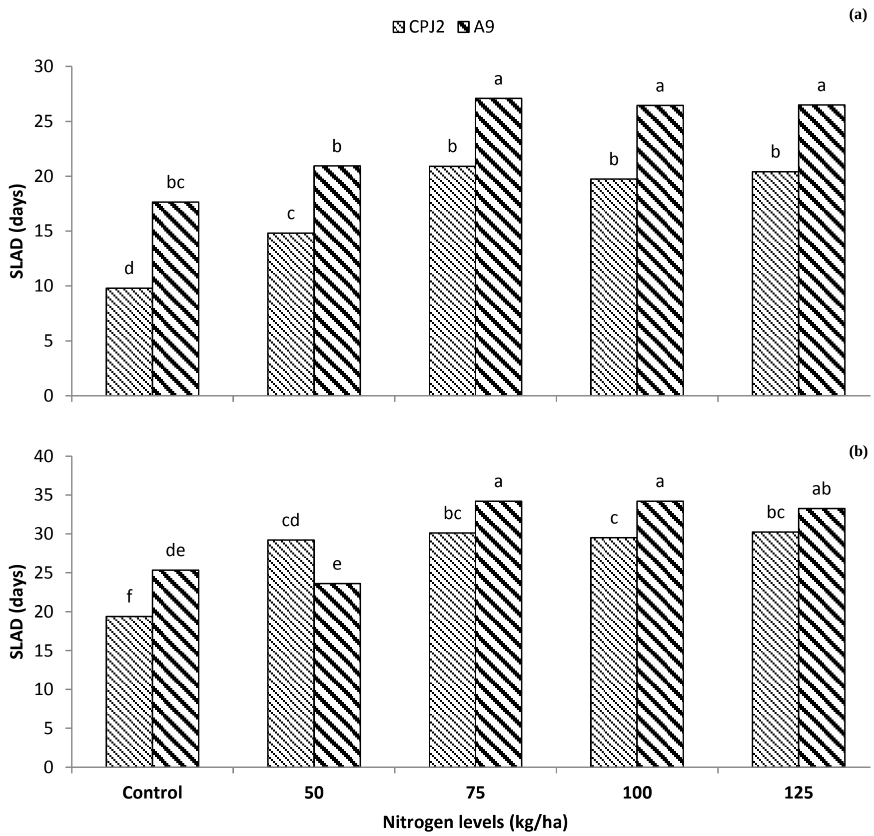


**Fig. 3:** Influence of nitrogen application on seasonal leaf area duration (days) before (a) and after (b) panicle emergence stage of two quinoa genotypes

phenology, much greater than expected from the general rule that low temperatures lead to a later and high temperatures to an earlier onset of phenological phases (Jochner, 2011). Days to attain physiological maturity were affected by N in quinoa genotypes. The reason may be that the N helps the crop to stay green by maintaining leaf chlorophyll content and stomatal opening, ultimately delaying senescence and more translocation of assimilates from leaf to seed which extend grain filling period, ultimately delays the physiological maturity. Many investigators observed these phenomena in wheat crop (Uhart and Andrade, 1995; Singh and Wilkens, 1999).

Nitrogen supplementation increased all growth related traits like crop growth rate, leaf area index, plant height, stem diameter, leaf area duration. The basis of these increments seems due to fact that N is an integral part of photosynthetic machinery (chlorophyll molecule and chloroplast) (Hak *et al*., 1993), which is conversion unit of light energy to chemical energy of photosynthetic apparatus. More chlorophyll, increased photosynthetic active leaf area, more will be assimilation resulting in better growth and development (Evans, 1983; Daughtry *et al.*, 2000; Amaliotis *et al.*, 2004; Ványiné *et al.*, 2012). Nitrogen supply also increases rates of cell division and expansion (Roggatz *et al*., 1999), photosynthesis and leaf production (Zhao *et al.*, 2003, 2005a, b). Nitrogen affects chlorophyll concentration of leaf which results in improved photosynthetic efficiency and outcome is in the form of improved and completion of early vegetative growth phases (Amaliotis *et al*., 2004).

The present investigation revealed that nitrogen fertilization can improve yield of quinoa under local conditions. In this experimentation nitrogen fertilization especially moderate dose of 75 kg ha$^{-1}$ N maximally improved main panicle length, number of panicle per plant, economic harvest and biomass production in both quinoa genotypes under study (Table 2). Increased number of panicle per plant due to N fertilization indicates involvement of N in increasing the production and distribution of photoassimilates from source (vegetative part) to sink (reproductive parts) (Muchow, 1988). This source sink relationship is due to development and maintenance of leaf area which is influenced by N (Arduini *et al*., 2006) as a result photosynthetic efficiency as well as partitioning of dry matter to reproductive organs improved during optimum nitrogen conditions (Prystupa *et al*., 2004). Fertilization of nitrogen (75, 100 and 125 kg N ha$^{-1}$) significantly improved main panicle length of quinoa genotypes, which is associated with number of grains longer panicles produce maximum number of grains. Nitrogen deficiency limits cell division and expansion, leaf production, photosynthesis as

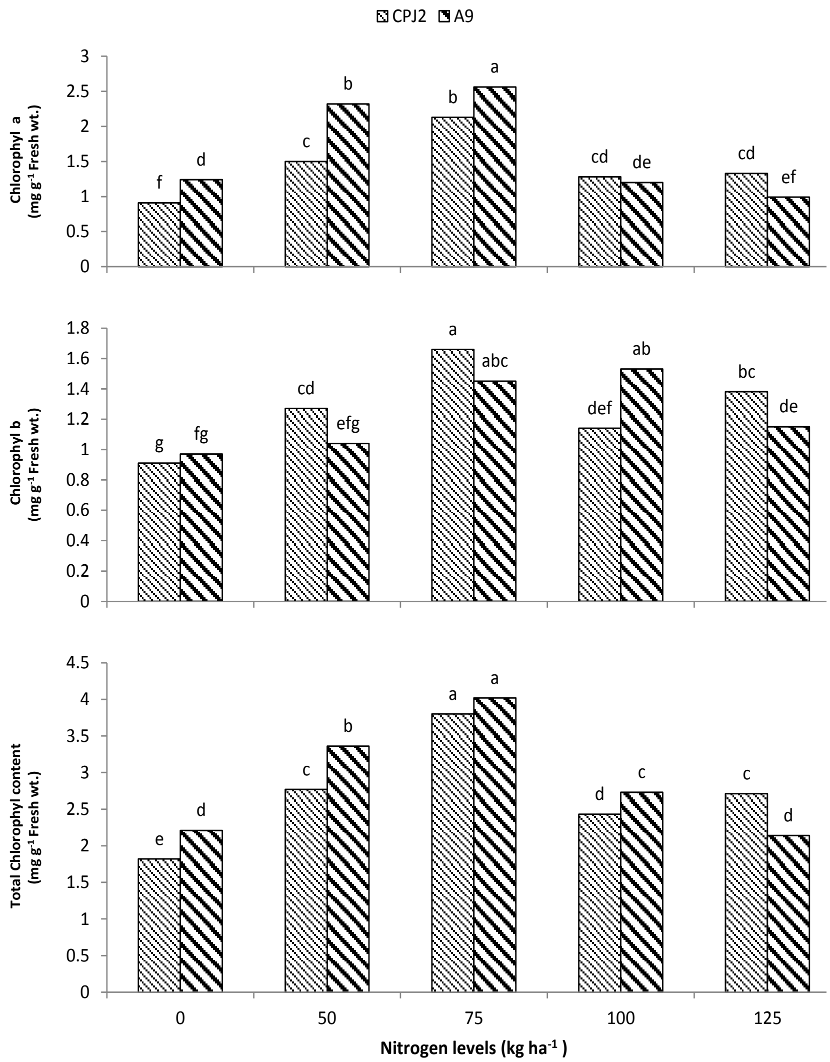


**Fig. 4:** Influence of nitrogen chlorophyll contents of both quinoa genotypes

well as seed yields (Zhao *et al*., 2003, 2005a, b). Nitrogen did not affect grain weight (thousand grain weight) of quinoa genotypes, however little difference was observed in genotypes CPJ2 and A9. It might be due to genetic difference of genotypes. No doubt, grain yield was significantly increased with N application but percent increase (145%) over control was recorded in genotype CPJ2 at 75 kg N ha$^{-1}$ and percent increase (166%) over control was observed at 100 kg N ha$^{-1}$ in genotype A9 (Table 2). The increase in biological yield by nitrogen treatments might be due to improved leaf area development and photosynthetic efficiency (Arduini *et al*., 2006) due to fact that N is a part of Rubisco (Fredeen *et al*., 1991).

Light harvesting pigments of photosynthesis chlorophyll a, chlorophyll b and total chlorophyll contents increased by increasing N levels in both genotypes (Fig. 4). Similar trend was also reported by Ványiné *et al.* (2012) due to varying level of N fertilization (0, 30, 60, 90, 120 and 150 kg N ha$^{-1}$). Leaf nitrogen is affected by N fertilizer which is directly proportional to chlorophyll content of leaf (Evans, 1983). Many researchers verified the strong linkage between Nitrogen content of leaf and chlorophyll (Evans, 1983; Amaliotis *et al.*, 2004). It is a well-known fact that N is a structural component of protein and chlorophyll molecules, thus determines the creation of chloroplast because 75% of leaf nitrogen is consumed in its formation (Hak *et al.*, 1993) and also accretion of chlorophyll in it (Daughtry *et al.*, 2000).

It can be concluded that quinoa is highly responsive to nitrogen fertilizer. Nitrogen level of 75 kg N ha$^{-1}$ was proved to be best level for N supplementation of soil for quinoa growth and development to harvest maximum economic harvest under agroecological conditions of Faisalabad. Genotype A9 yielded much more as compared to CPJ-2 due to an improvement in yield related traits.

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