*Pak. J. Bot.*, 40(4): 1681-1491, 2008.

# QUANTIFICATION OF PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN RICE DUE TO PLANTING MODES

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**Abstract**

The objectives of the research project were to evaluate the effect of planting modes based on varying moisture levels on the growth and yield of two lowland rice cultivars viz. KS-282 (coarse cultivar) and BAS-385 (fine cultivar) under natural conditions at three successive stages (viz. tillering, soft dough and mature grain stages) of grain filling. The two cultivars were grown in two different planting modes i.e. beds and flat fields at Kala Shah Kaku (KSK), Lahore. The endogenous level of growth promoting hormones Gibberellic Acid (GA) and Indole Acetic Acid (IAA) as well as sugar and protein content of leaves and grain increased at tillering and soft dough stages but decreased at mature grain stage. The magnitude of increase was more in flat field (FF) as compared to raised beds (RB) in both the cultivars. The stomatal resistance of flag and penultimate leaves was higher in flat field as compared to raised beds at tillering stage in both the cultivars. Root length, weight and plant height was significantly higher in BAS-385 as compared to KS-282 under both planting modes. It was concluded that difference in the moisture availability significantly affected the physiology of plants leading to higher yield in FF planting modes as compared to RB. The endogenous level of plant hormones (IAA and GA) affected the translocation of assimilates. KS-282 cultivar performed better with respect to yield. However, leaves and grains of BAS-385 contained more sugar and protein content than KS-282.

## Introduction

Rice production in Asia needs to be increased in order to feed an ever-growing population. More than 75% of the rice produced comes from irrigated land. However, the water crisis threatens the sustainability of the irrigated system. The supply of water for irrigation is endangered by declining water availability and quality, and increased competition from other users. Rice is cash crop in Pakistan and accounts for 5.7% of value added in agriculture and 1.2% in Gross Domestic Product (GDP) (GOP, 2007). It grows in soil with moisture regime that ranges from the submerged lowland to the water deficient upland and with nutrient transformation processes that varies with moisture regimes (Data, 1981).

Rice crop can be grown in different locations and under variety of climate and soil moisture conditions. The variation in soil moisture can cause numerous physiological and biochemical changes in various plant parts to sense soil water status. This variation in soil moisture influences rice yield by directly affecting the physiological process involved in vegetative growth and grain production. Intermittent flooding and drying of the soils depresses availability of several nutrients for rice uptake, and low nutrient supply will limit the potential yield (Bell *et al*., 2001). Bano *et al*., (1993) revealed that when rice was subjected to drought, root signals have been shown to regulate transpiration and leaf extension before shoot water status is affected. Changes in chemical composition of the

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xylem sap and changes in the plant water status have been shown to act as signals in root-to-shoot communication under stress (McDonald & Davies, 1996). Concentration of plant hormones in xylem sap has been shown to be the most important signal controlling stomatal resistance (Borel *et al*., 1997), which plays a key role in reducing water loss under extreme drought stress conditions.

Present investigation was aimed to study the effect of planting modes on yield with emphasis on monitoring the changes in the endogenous level of phytohormones, sugar and protein in leaves and grains of rice concomitant with the changes in stomatal resistance of flag and penultimate leaves.

## Materials and Methods

The study was conducted jointly by Rice Programme, NARC and Quaid-I-Azam University. A coarse (KS-282) and a fine (BAS-385) rice cultivars were grown in two different planting modes i.e. raised beds (RB) and flat fields (FF) at Rice Research Institute Kala Shah Kaku (KSK). The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications at 31.48.00 N latitude, 73.49.00 E longitude and 227 m elevation. Plot size was 10m x 20m. The plant-to- plant and row-to-row distance were kept at 20 cm in flat fields. Raised beds were 75 cm wide having two rows 20 cm apart from each other on each bed. The rice samples were collected at tillering, grain filling and maturity stages. Leaves and spikes were subsequently analyzed for sugar according to Dubo *et al*., (1956) as modified by Johnson *et al.* (1966) and protein content was determined following Lowery *et al.,* (1951). Extraction and purification of IAA and GA from leaves and spikes were made following the method of Kettner & Doerffling (1995). HPLC analyses of these hormones were made according to Li *et al*., (1994).

## Results and Discussion

There were significant differences in endogenous level of GA and IAA in both leaves and grains of two cultivars during its growth under two planting modes. GA and IAA content increased markedly (36%) & (52%) in leaves of BAS-385 (Table 1) and (23%) & (42%) in KS-282 cultivars at soft dough stage while in grains it was increased up to (39%) & (13%) in BAS-385 and (44%) & (13%) in KS-282 respectively (Table 2). GA and IAA contents decreased remarkably in leaves as well as in grains at mature grain stage under both FF & RB planting modes but the magnitude of decrease was greater in RB as compared to FF growing conditions in both the cultivars. As lowland rice is grown in bunded fields, where the soil conditions ranging from anaerobic to aerobic during crop growth, this might be the reason for greater GA in FF planting mode at soft dough stage (Wade *et al*., 1998). Rice plants develop their root system under anaerobic flooded conditions, which results in different expressions of root anatomy and gross root morphology from those under an upland aerobic environment.

This increase in grain GA and IAA may be attributed to rapid transport of growth promoting hormones from vegetative organs to the developing grains at soft dough stage. GA content in rice grains was high at early grain filling stage reaching maximum at 6-9 days post anthesis and then declined markedly (Qin & Tang, 1984; Yang *et al*., 2001).

**Table 1: GA and IAA contents (ng/g) in leaf at tillering soft dough and maturity of rice grown in field under natural climatic conditions. Figures in parentheses show the values of standard error.**

| Planting modes | BAS- 385 | | KS-282 | |
|---|---|---|---|---|
| | GA | IAA | GA | IAA |
| | | Tillering | | |
| **Flat Field** | 18 ( ± 0.33) | 9b (± 0.07) | 16b (±0.17) | 10a ( ± 0.10) |
| **Raised Bed** | 13c ( ± 0.31) | 6c ( ± 0.15) | 10d ( ± 0.12) | 6c ( ± 0.02) |
| **Mean** | 15.21 | 7 | 14 | 8 |
| | LSD = 0.60<br>CV = 2 % | LSD = 0.41<br>CV = 3 % | | |
| | | Soft dough | | |
| **Field** | 25a ( ± 0.12) | 17b ( ± 0 .12) | 17b ( ± 0.25) | 21a ( ± 0.12) |
| **Raised Bed** | 16c( ± 0.25) | 8d ( ± 0.15) | 12d ( ± 0.75) | 11c ( ± 0.12) |
| **Mean** | 21 | 12 | 14 | 19 |
| | LSD = 2<br>CV = 5% | LSD = 0.38<br>CV = 1 % | | |
| | | Maturity | | |
| **Field** | 12a ( ± 0.17) | 11b ( ± 0.08) | 9b ( ± 0.07) | 12a ( ± 0.10) |
| **Raised Bed** | 8c ( ± 0.05) | 8d ( ± 0.05) | 7d ( ± 0.05) | 8c ( ± 0.06) |
| **Mean** | 10 | 10 | 8 | 10.02 |
| | LSD = 0.36<br>CV = 27 % | LSD = 0.15<br>CV = 0.80 % | | |

**Table 2: GA and IAA contents (ng/g) in grains at soft dough and mature grains stage of rice grown in field under natural climatic conditions. Figures in parentheses show the values of standard error.**

| Planting modes | BAS- 385 | | KS-282 | |
|---|---|---|---|---|
| | GA | IAA | GA | IAA |
| | | Soft dough | | |
| **Flat Field** | 38 (± 0.22) | 15c ( ± 0.10) | 36a ( ± 0.50) | 21a ( ± .10) |
| **Raised Bed** | 24b ( ± 1.73) | 13d ( ± 0.20) | 20c ( ± 0.25) | 16.50b ( ± 0.12) |
| **Mean** | 31 | 14 | 28 | 19 |
| | LSD = 4<br>CV = 6 % | LSD = 0.51<br>CV = 2 % | | |
| | | Mature grain | | |
| **Flat Field** | 12a ( ± 0.27) | 7a ( ± 0.15) | 6c ( ± 0.07) | 8a ( ± 0.20) |
| **Raised Bed** | 8b ( ± 0.27) | 4c ( ± 0.15) | 4d ( ± 0.28) | 4d ( ± 0.15) |
| **Mean** | 10 | 5 | 5 | 6 |
| | LSD = 0.40<br>CV = 3% | LSD = 0.09<br>CV = 0.09 % | | |

**Table 3: Protein and sugar contents (mg/g) in leaf at tillering soft dough and maturity of rice grown in field under natural climatic conditions. Figures in parentheses show the values of standard error.**

| Planting modes | BAS- 385 | | KS-282 | |
|---|---|---|---|---|
| | Protein | Sugar | Protein | Sugar |
| | | Tillering | | |
| Flat Field | 362a (± 5) | 105ab (± 3) | 343ab (±11) | 107a (± 4) |
| Raised Bed | 332b (± 6) | 95ab ( ± 3) | 288c (± 4) | 93b (± 4) |
| Mean | 347 | 100 | 316 | 100 |
| | LSD = 22<br>CV = 3% | LSD = 12<br>CV = 6% | | |
| | | Soft dough | | |
| Flat Field | 443a (± 12) | 120a ( ± 3) | 430ab (± 9) | 110b (±3) |
| Raised Bed | 403b (± 8) | 95c ( ± 3) | 430ab (± 9) | 90c (±3) |
| Mean | 423 | 108 | 430 | 100.00 |
| | LSD = 28<br>CV = 3 % | LSD = 10<br>CV = 5% | | |
| | | Maturity | | |
| Flat Field | 328b (± 5) | 112a ( ± 5) | 305a (± 9) | 100.00ab + 2 .5) |
| Raised Bed | 292c (± 9) | 85bc ( ± 3) | 280a (± 9) | 75.00c ( ± 5) |
| Mean | 310 | 98 | 292 | 87.500 |
| | LSD = 14<br>CV = 2 % | LSD = 18<br>CV = 10 % | | |

**Table 4: Protein and sugar contents (mg/g) in grains at soft dough and mature grains stage of rice grown in field under natural climatic conditions. Figures in parentheses show the values of standard error.**

| Planting modes | Soft dough | | | |
|---|---|---|---|---|
| | BAS- 385 | | KS-282 | |
| | Protein | Sugar | Protein | Sugar |
| Flat Field | 195a (± 10) | 85a (± 3) | 185a (± 9) | 73b ( ± 4) |
| Raised Bed | 170a (± 8) | 53c ( ± 4) | 183a (± 17) | 40d ( ± 3) |
| Mean | 183 | 69 | 184.16 | 57 |
| | LSD = 00<br>CV = 5% | LSD = 12<br>CV = 9 % | | |
| | | Mature grain | | |
| Flat Field | 152a (± 12) | 25a ( ± 3) | 180a (± 8) | 25a ( ± 3) |
| Raised Bed | 138a (± 13) | 20a ( ± 3) | 157a (± 21) | 20a ( ± 3) |
| Mean | 145 | 23 | 169 | 23 |
| | LSD = 65<br>CV = 21 % | LSD = 11<br>CV = 25% | | |

**Table 5: Plant height, panicle size (cm) and number of productive tillers/plant at mature grain stage of rice crop grown in field under natural climatic conditions. Figures in parentheses show the values of standard error.**

| Parameters | Flat Field | | | |
|---|---|---|---|---|
| | BAS- 385 | KS-282 | LSD | CV |
| **Plant Height** | 130a ( ± 1) | 99b ( ± 0.50) | 4 | 2 |
| **Panicle Size** | 28a ( ± 1) | 28a ( ± 0.50) | 1 | 2 |
| **Productive Tillers** | 15a ( ± 0.50) | 12c ( ± 0.50) | 1 | 5 |
| **1000 Grain Weight** | 21c (± 0.10) | 22a ( ± 0.10) | 0.23 | 0.6 |
| **Yield** | 4120c ( ± 0.50) | 6008a ( ± 0.50) | 0 | 3 |
| | **Raised Bed** | | | |
| **Plant Height** | 128a ( ± 1.00) | 93.00c ( ± 1.00) | 4 | 2 |
| **Panicle Size** | 28a ( ± 1.00) | 24.00b ( ± 0.50) | 1 | 2 |
| **Productive Tillers** | 14b ( ± 0.50) | 10d ( ± 0.50) | 1 | 5 |
| **1000 Grain Weight** | 20d (± 0.50) | 21b ( ± 0.50) | 0.23 | 0.6 |
| **Yield** | 3787d ( ± 0.50) | 4775b ( ± 0.50) | 0 | 3 |

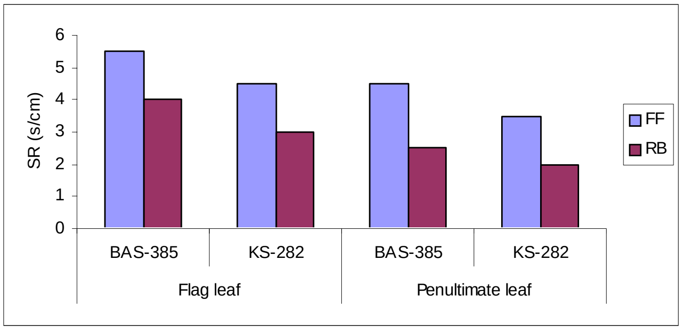


**Fig. 1: Stomatal resistance (s/cm) in flag and penultimate leaf at tillering stage of rice crop rice grown in field under natural climatic conditions.**

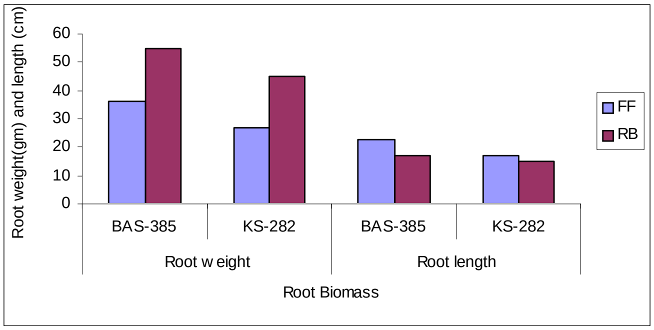


**Fig. 2: Root weight (g) and root length (cm) at tillering stage of rice crop grown in field under natural climatic conditions.**

Kende & Zeevaart (1997) and Hansen and Grossmann (2000) reported that the process of grain development is regulated by auxin and gibberellin. Similarly, Kende and Zeevaart, (1997) concluded that auxin stimulates cell division in combination with cytokinin and its content increased at early grain filling stage (Morris *et al.*, 1993). IAA in the grains may regulate cell division in rice endosperm and thus the sink size of the grain at early grain filling stages, (Yang *et al*., 2003).

Exposure of the root system of plant to water logging conditions, according to Letham *et al*., (1978), cause fluctuation in the hormonal balance. Under water logged condition increase in GA content might be caused by adventitious root formation and root may be site for gibberellin biosynthesis (Jones & Phillips, 1966) or a locus for the interconversion of one gibberellin to another. Phytohormones may also enhance root development under both FF as well as RB growing conditions. Kuroha *et al*., (2002) reported that plant hormones stimulate adventitious root formation. Similar findings have been reported previously (Oda *et al*., 2003). The ability of the plants to absorb nutrients efficiently from the soil can be attributed to higher number of adventitious roots and GA promotes (Wahyuni *et al*., 2003; Kono, 1995; Watanabe, 1997). Root initiation, adventitious root formation, and early development of root are also stimulated by auxin (Bellamine *et al*., 1998; Pan & Tian, 1999). Waterlogged condition in the soil stimulated adventitious root initiation (Colmer *et al*., 2003).

There was significant increase in endogenous level of GA and IAA in xylem sap under both the planting modes in both cultivars; however the magnitude of increase was higher (50%) at FF as compared to RB growing conditions at tillering stage (Fig. 1). In controlling stomatal resistance, the most important signal is considered to be the concentration of plant hormones in xylem sap (Borel *et al*., 1997). Root-produced organic compounds in xylem sap, such as hormones and amino acids, are considered to be vital for plant development (Oda *et al*., 2003).

Root weight significantly differs in both the cultivars with respect to planting modes FF vs. RB growing conditions at tillering stage. BAS-385 had higher root weight under both the growing conditions but the magnitude of increase was higher in RB of BAS-385 as compared to KS-282 (Fig. 2). Root weight was increased to (52%) in BAS-385 & (66%) in KS-282 under RB growing conditions as compared to FF. BAS-385 showed (26%) increase in root length in FF conditions, whereas in KS-282 it was (12%) higher as compared to RB (Fig. 2). Kusnarta *et al*, (2004) concluded that root distribution of plants is controlled by porosity, compaction, water content and concentration of organic matter, which in turn are affected by the system of soil management (unflooded raised beds or flooded flat land, with and without tillage). Roots play a significant role in regulating the growth of above ground parts in plants (Davies & Zhang 1991), and closely correlated with photosynthesis in leaves (Zhai *et al*., 2002). Roots are normally shallow in rainfed lowlands rice (Pantuwan *et al*., 1997); differences in root growth and development are known to occur among different genotypes in deeper layers (Samson & Wade, 1998). Despite having fewer roots in deeper layers, rainfed lowland rice can extract water from below a 15-cm soil depth (Wade *et al*., 1999).

Protein content of leaves and grains increased significantly at soft dough stage under both growing conditions, but the magnitude of increase was higher in FF as compared to RB in both the cultivars. BAS-385 accumulated greater amount of protein as compared to that of KS-282. Protein content increased markedly (9%) in leaves of BAS-385 under FF growing conditions at soft dough stage, while in grains it was increased upto (13%) &

(1%) in BAS-385 and KS-282 cultivar respectively (Table 4) . There was marked decrease of protein content in leaves as well as in grains at mature grain stage in both planting modes, however the magnitude of decrease was more in RB of KS-282 as compared to BAS-385. This may be due to influence of growth promoting hormones (IAA, GA) which are likely to influence by enhancing enhanced enzyme activity which results in an increase in metabolic compounds (Woodword & Bartel, 2005; Peer *et al*., 2004). Low oxygen selectively induces the synthesis of proteins known as anaerobic proteins, majority of among which are enzymes involved in sugar metabolism, glycolysis, and fermentation processes (Sachs *et al*., 1980; Chang *et al*., 2000; Huang *et al*., 2005). Cultivar (genotype), management, and by environmental conditions have effect on protein levels.

It was also observed that greater sugar content was found in leaves (flag and penultimate) and grains of BAS-385 at soft dough stage under FF as compared to RB growing conditions. Sugar content increased markedly (21%) & (18%) in leaves of BAS-385 and KS-282 cultivars under FF growing conditions at soft dough stage, while in grains it was increased up to (60%) & (45%) respectively. Water stress induced the accumulation of sugar in leaves of mung bean plants (Farooq & Bano, 2006). There was marked decrease of sugar content in leaves as well as in grains at mature grain stage in both planting modes, however the magnitude of decrease was more in RB of KS-282 as compared to BAS-385. The decrease in sugar content on RB as compared to the FF may be due to osmotic stress a plant may encounters under raised bed conditions, which induce in plant a strategy to confer stress tolerance in the form of accumulation of sugar. Cultivar (genotype) may differ in their sugar content and it might also be influenced by, management and by environmental conditions. Sugar is the principal and major storage form of photoassimilate in leaves (Koch, 1996). Stimulation of enzyme synthesis for mobilization of seed reserves in germinating grains is thought to be caused by GA, which is also known to stimulate growth of intact plants (Salisbury & Ross, 1992; Arteca, 1995; Johri & Mitra, 2001).

Stomatal resistance differed significantly in flag and penultimate leaf of both the cultivars with respect to planting modes FF vs. RB growing conditions at tillering stage. BAS-385 had higher stomatal resistance in both flag (27%) as well as penultimate leaf (44%) under FF planting mode as compared to RB while stomatal resistance was increased markedly (33%) in flag leaf and (50%) in panultimate leaf under FF conditions in KS-282 at tillering stage. BAS-385 had higher stomatal resistance in both flag as well as penultimate leaf at FF and RB growing conditions as compared to that of KS-282. This increase of stomatal resistance in FF may possibly be attributed to the fact that under water logged conditions the root tips get slugged off resulting in decreased uptake of nutrients and water thus disturbing the water balance of the plant hence stomatal resistance gets higher to maintain water budget. This is reflected in the greater root biomass in RB as compared to FF planting modes in both the cultivars. Among the signals controlling stomatal resistance, concentration of plant hormones in xylem sap is most important (Borel *et al*., 1997). Increase in evaporative demands also result in an increase in the pH of leaf sap, which can promote ABA accumulation and lead to reduction in stomatal conductance (Wilkinson & Davies, 2002; Davies *et al*., 2002). Ali *et al*. (2007) and Aslam *et al.* (2006) observed marked reduction in stomatal conductance in maize plant under water stress conditions.

Variation in plant height was observed in the two cultivars, however plant height was significantly different in KS-282 as compared to BAS-385 at mature grain stage under both planting modes. There was marked increase in plant height (2% & 6%),productive tillers (10% & 34%) and grain yield (8% & 21%) in BAS-385 and KS-282 respectively under FF conditions as compared to RB. 1000 grain weight was increased up to (4%) in BAS-385 and (4%) in KS-282 under FF planting modes (Table 5). There were differences in number of tillers, 1000 grain weight and yield between two planting modes of BAS-385 and KS-282. Higher yield was recorded in KS-282 as compared to BAS-385 at maturity under both growing conditions; however magnitude of increase in grain yield was greater in FF as compared to RB growing conditions. This increase may be due to effect of growth promoting hormones (IAA, GA), which was found to be higher in FF as compared to RB planting modes. Involvement of auxin and gibberellin in regulating grain development is reported (Hansen & Grossmann, 2000). Wang *et al*, (1998) and Yang *et al*, (1999) suggested that reduced IAA content in rice grains could be a cause of poor grain filling.

Variation in soil moisture may influences rice yield by directly affecting the physiological process involved in vegetative growth and grain production. Irrigated rice yield declines as soon as the field water content drops below saturation (Bouman & Tuong, 2001). By planting one seedling per hill, Wen & Yang (1991) obtained higher rice yield, effective panicles, number of grains panicle$^{-1}$ and 1000-grain weight. Singh (1994) noted that the number of grains and grain weight panicle$^{-1}$ had a positive correlation with grain yield. Maqsood & Azam (2007) investigated that drought stress reduced seed weight and yield in finger millet plants.

**Conclusion:** It was concluded that planting mode significantly affected the growth of rice plant. It is evident that FF planting mode resulted in greater production of Phytohormones (IAA and GA) in plants than that of RB resulting in greater yield in FF than RB. Results also revealed that greater translocation of assimilate mainly sugar and protein contents from rice leaves to grains was found under FF condition as compared to RB. Results also suggested that FF is better planting modes with respect to yield, sugar and protein translocation and endogenous hormonal level than RB due to the differences in moisture availability in the two planting modes.

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(Received for publication 21 April, 2008)