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# BIOCHEMICAL CHARACTERIZATION OF RICE (*ORYZA SATIVA* L.) FOR FOSTERING PRODUCTIVITY UNDER MOISTURE DEFICIT CONDITIONS

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**Abstract**–Two sets of experiments were conducted one in laboratory, and the other in field during Rabi season 2011 & 2012-13 with 5 rice varieties viz. Mandakini, SahabhagiDhan, Parijat, Annada& Anjali in Chiplima farm to assess the efficiency and efficacy of moisture stress for their relative tolerance. The field studies were conducted taking 35 treatments laid out in split plot design with 3 replications with 7 combinations of drought stress comprising stress at 3 different stages of crop growth i.e. tillering, panicle initiation and flowering. Chl-a,Chl-b, Total Chlorophyll content and CSI also decreased on account of moisture stress in all the varieties. The reduction in value of these characters was lower in tolerant varieties compared to the susceptible ones. When the moisture stress was imposed the NR activity decreased irrespective of the stage of the growth and the varieties. The tolerant variety Anjali recorded the highest NR activity in stress conditions compared to others. In contrast the proline content was found to accumulate more in case of susceptible varieties than the tolerant varieties.

## INTRODUCTION

Rice is one of the world's most important crops in terms of economic value and 90% of the world's rice is grown and consumed in Asia. Rice is the dominant staple food of the country and it holds the key for food security. It occupies 44 million ha with a production of 112 million tons in Eastern India comprising 26.8 million ha of which 14.7 million ha, are under rain fed lowlands. Rice being the main stay of 50 % of the global population bears a testimony to its importance while planning for food security of the ever-burgeoning population of the world. Almost half of the world's rice about 45% is grown in rainfed ecosystem where production is dependent on a well and evenly distributed rainfall (Haefele *et al.,* 2016). Rice production in Asia needs to be increased to feed the growing population. Albeit, a complete assessment of the level of water scarcity in Asian rice production is still lacking, there are signs that declining quality as well as declining availability of water resources are threatening so far as the sustainability of the irrigated rice-based production system is concerned. Water deficit and survival rely on a series of mechanisms that can be grouped as avoidance or tolerance mechanisms.

Plants respond to drought at physiological and molecular levels which lead to modification in plant metabolome and transcriptome. Stress factors such as drought trigger common reactions in plants and lead to cellular damages mediated by innocuous reactive oxygen species (Mano *et al.,* 2002). Accumulation of reactive oxygen species (ROS) induces oxidative damage to proteins, membrane lipids and other cellular components (Jimenez *et al.,* 2002). Adaptation or acclimation to adverse habitat, edaphic or environmental, is a common feature of plant kingdom. These adaptations are achieved through varied molecular, cellular, biochemical, and physiological, anatomical and morphological modifications in plants (Bohnert *et al.*, 1995). The rice plant responds to the moisture deficit conditions in various ways. Although drought is a major problem for rice grown under rain fed lowland and upland conditions, progress in breeding to improve drought resistance has been slow. This study was undertaken with the main objective to have a greater insight into this physiological and biochemical basis of drought tolerance in rice which would come in handy in designing the crop ideotypes for drought prone environments and formulating a strategy to ameliorate the effect of stress suitably so as to minimize the yield loss at times of water deficit. Moreover, the present study aims to ransack the response of rice plants to drought under agro-climatic conditions of Odisha with the objectives, to identify changes in some of the senescence markers like chloroplast pigments, NR-proteins activity etc. and to identify measures for overcoming the innocuous effects of drought by improving the degree of resistance in the plants through drought mitigating chemicals.

## MATERIALS AND METHODS

### Plant Materials

The biochemical basis of drought tolerance in rice was studied using two sets of experiments, one in the laboratory and other in the field conditions. Five varieties of rice, viz. *Annada, Anjali, Mandakini, Parijat, Sahabhagi Dhanwere* taken for the study. Required quantities of seeds were collected from NRRI, Cuttack and Central farm OUAT for the above purpose.

### Biochemical Parameters Estimation

Different biochemical studies were taken up during the crop growth period as well as after the crop was harvested. Chlorophyll-a, chlorophyll-b and total chlorophyll content in the leaves were determined by using the standard method (Arnon, 1949). Chlorophyll Stability Index (CSI) was calculated by taking leaf samples of control as untreated and those imposed with drought stress as treated and using the standard formula (Chauhan *et al.,* 2005). Nitrate reductase (NRA) activity was determined by using a standard procedures (Hatam and Hume, 1976). Proline accumulation was determined by estimating free proline content in the leaves with slight modifications (Bates *et al.,* 1973; Sadasivam and Manickam, 1992). The quantity of free proline was calculated from a standard curve prepared from purified proline. Proline accumulation was expressed as micrograms of proline per gram fresh weight of leaf tissue (μg proline $g^{-1}$ FW).

## RESULTS

### Chlorophyll Content

The chlorophyll content of leaf estimated at different stages of growth have been presented in Table 1, Table 2 and Table 3. Irrespective of varieties it was noted that the chlorophyll content was highest at tillering stage and decline at panicle initiation stage and thereafter, chlorophyll-a content was highest at tillering stage and thereafter declined till panicle initiation stage. The chlorophyll a content decreased due to water stress but salicylic acid application on stress increased the value by 30.7%. Annada recorded the highest mean chlorophyll content 1.89 mg/g FW of leaf at tillering, 1.84 mg/g FW of leaf at PI stage and 1.5084 mg/g FW of leaf at flowering stage. Parijat recorded the lowest chlorophyll -a values for all stages with 0.99, 0.98 and 1.18 mg/g fresh weight of leaf at tillering,PI and flowering stages respectively. Across the growth stages similar trend in chlorophyll–b content was recorded. Due to stress a decrease of chlorophyll b content was observed but application of salicylic acid significantly increased the chlorophyll b content. Annada recorded highest chlorophyll b content at all stages of growth whereas the lowest value was observed in Parijat. Due to exposure to stress all the genotypes experienced decrease in total chlorophyll content like chlorophyll a and chlorophyll b. In response to stress there was decrease of total chlorophyll content to a tune of 22%, 32% and 54% at tillering, panicle initiation and flowering stage as compared to control respectively. The average highest total chlorophyll content was found in Annada followed by Anjali but the lowest value of the same was recorded from Parijat.

### Chlorophyll Stability Index

Data on chlorophyll Stability Index (CSI) presented in Table 4 revealed that it decreased with increase of moisture stress irrespective of genotypes and stages of growth. At tillering stage when moisture stress was imposed the highest decrease was recorded in Parijat (45%) followed by Sahabhagi Dhan (36%). whereas the lowest value of the same was noted from Anjali (33%). Application of SA has significant impact on the varieties. Similarly at PI stage when moisture stress was imposed a decrease in

**Table 1.** Effect of moisture stress on Chl-a(mgg-[1]FW of tissue) at different growth stages of rice cultivar

| Treatment | Tillering | | | PI stage | | | Flowering stage | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2011 | 2012 | Pooled | 2011 | 2012 | Pooled | 2011 | 2012 | Pooled |
| T1 | 2.28 | 2.54 | 2.41 | 1.78 | 1.95 | 1.86 | 1.30 | 1.46 | 1.38 |
| T2 | 1.58 | 1.76 | 1.67 | 1.26 | 1.38 | 1.32 | 1.04 | 1.17 | 1.10 |
| T3 | 1.80 | 2.0 | 1.90 | 1.62 | 1.78 | 1.70 | 1.18 | 1.32 | 1.25 |
| T4 | 1.98 | 2.19 | 2.10 | 1.06 | 1.161 | 1.11 | 0.90 | 1.01 | 0.96 |
| T5 | 1.95 | 2.16 | 2.05 | 1.00 | 1.09 | 1.04 | 1.18 | 1.32 | 1.25 |
| T6 | 1.56 | 1.73 | 1.64 | 1.14 | 1.24 | 1.19 | 1.20 | 1.34 | 1.27 |
| T7 | 1.48 | 1.64 | 1.56 | 1.10 | 1.20 | 1.15 | 1.22 | 1.37 | 1.30 |
| Mean | 1.80 | 2.0 | 1.90 | 1.28 | 1.40 | 1.34 | 1.14 | 1.28 | 1.21 |
| SEm(±) | 0.035 | 0.054 | 0.031 | 0.023 | 0.034 | 0.021 | 0.023 | 0.034 | 0.021 |
| CD (0.05) | 0.099 | 1.2 | 0.094 | 0.064 | 0.076 | 0.060 | 0.065 | 0.067 | 0.060 |
| *Variety* | | | | | | | | | |
| Mandakini (V1) | 1.75 | 1.52 | 1.63 | 1.45 | 1.68 | 1.56 | 1.14 | 1.30 | 1.22 |
| SahabhagiDhan (V2) | 1.67 | 1.46 | 1.56 | 1.04 | 1.21 | 1.12 | 1.05 | 1.20 | 1.12 |
| Parijata(V3) | 1.01 | 0.88 | 0.99 | 0.91 | 1.06 | 0.98 | 1.10 | 1.26 | 1.18 |
| Annada(V4) | 2.02 | 1.76 | 1.89 | 1.61 | 1.87 | 1.74 | 1.41 | 1.60 | 1.50 |
| Anjali(V5) | 1.97 | 1.72 | 1.84 | 1.58 | 1.84 | 1.71 | 1.20 | 1.37 | 1.28 |
| Mean | 1.68 | 1.47 | 1.57 | 1.32 | 1.53 | 1.42 | 1.18 | 1.34 | 1.26 |
| SEm(±) | 0.035 | 0.045 | 0.028 | 0.025 | 0.034 | 0.057 | 0.023 | 0.036 | 0.020 |
| C.D (0.05) | 0.115 | 0.21 | 0.084 | 0.080 | 0.084 | 0.177 | 0.074 | 1.34 | 0.060 |

**Table 2.** Effect of moisture stress on Chl-b (mgg-[1]FW of tissue) at different growth stages of rice cultivar

| Treatment | Tillering | | | PI stage | | | Flowering stage | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2011 | 2012 | Pooled | 2011 | 2012 | Pooled | 2011 | 2012 | Pooled |
| T1 | 1.04 | 1.07 | 1.05 | 1.1 | 1.20 | 1.15 | 1.01 | 1.35 | 1.18 |
| T2 | 0.88 | 0.90 | 0.89 | 1.0 | 1.09 | 1.04 | 0.90 | 1.20 | 1.05 |
| T3 | 1.06 | 1.09 | 1.07 | 1.04 | 1.13 | 1.08 | 0.97 | 1.29 | 1.13 |
| T4 | 1.0 | 1.13 | 1.06 | 0.98 | 1.07 | 1.02 | 0.92 | 1.23 | 1.07 |
| T5 | 1.12 | 1.26 | 1.19 | 1.0 | 1.09 | 1.04 | 1.01 | 1.34 | 1.20 |
| T6 | 1.04 | 1.17 | 1.10 | 1.06 | 1.15 | 1.10 | 0.86 | 1.14 | 1.0 |
| T7 | 1.08 | 1.21 | 1.14 | 1.08 | 1.20 | 1.14 | 0.92 | 1.22 | 1.07 |
| Mean | 1.03 | 1.11 | 1.07 | 1.04 | 1.13 | 1.08 | 0.94 | 1.25 | 1.1 |
| SEm(±) | 0.033 | 0.035 | 0.02 | 0.018 | 0.034 | 0.018 | 0.007 | 0.009 | 0.005 |
| C.D (0.05) | 0.090 | 0.099 | 0.072 | 0.050 | 0.07 | 0.055 | 0.020 | 0.031 | 0.016 |
| *Variety* | | | | | | | | | |
| Mandakini (V1) | 7.58 | 7.62 | 7.60 | 1.04 | 1.06 | 1.05 | 0.91 | 1.10 | 1.00 |
| Sahabhagi Dhan (V2) | 7.52 | 7.57 | 7.54 | 1.01 | 1.04 | 1.02 | 0.88 | 1.11 | 0.99 |
| Parijata (V3) | 7.1 | 7.14 | 7.12 | 1.0 | 1.01 | 1 | 0.86 | 1.04 | 0.94 |
| Annada (V4) | 7.6 | 7.65 | 7.62 | 1.08 | 1.10 | 1.09 | 0.95 | 1.14 | 1.04 |
| Anjali (V5) | 6.76 | 6.80 | 6.78 | 1.01 | 1.03 | 1.02 | 0.93 | 1.12 | 1.02 |
| Mean | 7.31 | 7.35 | 7.33 | 1.03 | 1.049 | 1.036 | 0.91 | 1.10 | 1.002 |
| SEm(±) | 0.022 | 0.035 | 0.020 | 0.015 | 0.034 | 0.017 | 0.005 | 0.009 | 0.004 |
| C.D (0.05) | 0.069 | 0.078 | 0.060 | 0.048 | 0.056 | 0.059 | 0.017 | 0.028 | 0.014 |

chlorophyll stability index was resulted in all the varieties. The decrease was highest in Parijat (24.7%) followed by Sahabhagi Dhan whereas the lowest value of the same was observed in Anjali (13%). Effect of SA application was similar to tillering stage. The highest CSI was recorded from Annada (95.3 %); but when the varieties were exposed to moisture stress at flowering all the genotypes showed a reduction in their chlorophyll stability index. The decrease was highest in Sahabhagi Dhan and lowest in Annada (14%). Spaying of SA increased the CSI ranging from 2% in Annada to 10.6% in Sahabhagi Dhan. Among the varieties CSI was found highest in Anjali.

**Table 3.** Effect of moisture stress on total Chlorophyll (mgg-[1]FW of tissue) content at different growth stages of rice cultivar

| Treatment | Tillering | | | PI stage | | | Flowering stage | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2011 | 2012 | Pooled | 2011 | 2012 | Pooled | 2011 | 2012 | Pooled |
| T1 | 3.1 | 3.0 | 3.1 | 2.86 | 3.35 | 3.1 | 2.20 | 2.6 | 2.40 |
| T2 | 2.54 | 2.42 | 2.48 | 2.16 | 2.53 | 2.34 | 1.82 | 2.15 | 1.98 |
| T3 | 2.60 | 2.77 | 2.68 | 2.60 | 3.04 | 2.82 | 2.10 | 2.48 | 2.29 |
| T4 | 2.90 | 2.91 | 2.92 | 2.01 | 2.14 | 2.07 | 1.70 | 2.0 | 1.85 |
| T5 | 2.94 | 3.38 | 3.16 | 2.18 | 2.28 | 2.25 | 2.0 | 2.38 | 2.20 |
| T6 | 2.60 | 3.0 | 2.80 | 2.06 | 2.41 | 2.23 | 1.02 | 1.2 | 1.11 |
| T7 | 2.50 | 2.87 | 2.68 | 2.30 | 2.70 | 2.5 | 2.12 | 2.50 | 2.31 |
| Mean | 2.81 | 3.23 | 3.02 | 2.33 | 2.74 | 2.53 | 1.84 | 2.18 | 2.01 |
| SEm(±) | 0.050 | 0.07 | 0.04 | 0.054 | 0.057 | 0.039 | 0.056 | 0.067 | 0.043 |
| C.D (0.05) | 0.140 | 0.16 | 0.13 | 0.152 | 0.17 | 0.011 | 0.16 | 0.20 | 0.13 |
| *Variety* | | | | | | | | | |
| Mandakini (V1) | 2.85 | 3.05 | 2.95 | 2.5 | 2.94 | 2.72 | 2.12 | 2.5 | 2.31 |
| Sahabhagi Dhan (V2) | 2.71 | 2.90 | 2.81 | 2.04 | 2.4 | 2.22 | 1.80 | 2.12 | 1.96 |
| Parijata (V3) | 2.60 | 2.78 | 2.7 | 1.92 | 2.26 | 2.09 | 1.74 | 2.05 | 1.89 |
| Annada (V4) | 3.01 | 3.22 | 3.11 | 2.62 | 3.08 | 2.85 | 2.21 | 2.61 | 2.41 |
| Anjali (V5) | 2.95 | 3.16 | 3.1 | 2.58 | 3.04 | 2.81 | 2.10 | 2.48 | 2.29 |
| Mean | 2.82 | 3.02 | 2.92 | 2.33 | 2.74 | 2.53 | 1.99 | 2.35 | 2.17 |
| SEm(±) | 0.033 | 0.034 | 0.067 | 0.027 | 0.43 | 0.161 | 0.063 | 0.076 | 0.049 |
| C.D (0.05) | 0.106 | 0.107 | 0.201 | 0.087 | 0.098 | 0.48 | 0.206 | 0.231 | 0.147 |

**Table 4**. Effect of moisture stress on Chlorophyll Stability Index (%) at different growth stages of rice cultivar

| Treatment | Character | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Tillering | | | PI stage | | | Flowering stage | | |
| | 2011 | 2012 | pooled | 2011 | 2012 | pooled | 2011 | 2012 | Pooled |
| T1 | 99.28 | 100 | 99.64 | 97.06 | 98.7 | 98.0 | 99.66 | 99.5 | 99.6 |
| T2 | 63.86 | 65.14 | 64.5 | 92.58 | 94.4 | 93.3 | 97.98 | 97.9 | 97.9 |
| T3 | 81.36 | 83.0 | 82.2 | 94.88 | 96.7 | 95.7 | 97.8 | 98.0 | 98.0 |
| T4 | 98.0 | 98.1 | 99.05 | 77.2 | 78.7 | 78.0 | 88.64 | 89.0 | 88.8 |
| T5 | 99.7 | 99.1 | 99.4 | 86.28 | 88.0 | 87.01 | 92.34 | 92.0 | 92.2 |
| T6 | 98.5 | 97.0 | 97.7 | 98.7 | 97.6 | 98.15 | 87.38 | 88.0 | 87.7 |
| T7 | 98.2 | 97.5 | 97.8 | 99.7 | 99.0 | 99.3 | 87.44 | 88.5 | 88.0 |
| Mean | 91.27 | 91.4 | 91.33 | 92.6 | 94.4 | 93.5 | 93.03 | 93.3 | 93.2 |
| SEm(±) | 0.609 | 0.76 | 0.68 | 0.644 | 0.89 | 0.78 | 0.566 | 0.78 | 0.47 |
| C.D (0.05) | 1.722 | 1.82 | 2.05 | 1.22 | 1.45 | 2.33 | 1.602 | 2.45 | 1.42 |
| *Variety* | | | | | | | | | |
| Mandakini (V1) | 95.05 | 96.0 | 95.5 | 93.1 | 92.2 | 92.65 | 93.58 | 92.64 | 93.11 |
| Sahabhagi Dhan (V2) | 94.07 | 94.9 | 94.5 | 92.04 | 91.12 | 92.0 | 92.22 | 91.3 | 91.7 |
| Parijata (V3) | 90.42 | 91.32 | 91.0 | 89.91 | 89.01 | 89.5 | 9.52 | 90.60 | 91.0 |
| Annada (V4) | 93.0 | 94.0 | 93.5 | 92.37 | 91.5 | 91.9 | 91.45 | 90.54 | 91.8 |
| Anjali (V5) | 95.97 | 96.4 | 96.2 | 95.6 | 95.0 | 95.3 | 93.81 | 92.8 | 93.31 |
| Mean | 93.7 | 94.5 | 94.1 | 92.6 | 91.7 | 92.29 | 92.52 | 91.57 | 92.1 |
| SEm(±) | 0.434 | 0.567 | 0.35 | 0.421 | 0.67 | 0.38 | 0.550 | 0.87 | 0.503 |
| C.D (0.05) | 1.417 | 2.12 | 1.05 | 1.371 | 1.56 | 1.15 | 1.795 | 2.76 | 1.51 |

**Nitrate Reductase Activity (NRA activity)**

NR registered a decrease trend due to impact of moisture stress in all stages of growth irrespective of varieties (Table 5). The maximum reduction at tillering was 39% in Annada but Anjali recorded the highest NR activity at all stages of growth followed by Anjali. At tilering stage in Annada application of salicylic acid to stressed plants enhanced 6.4%

higher NR activity compared to the control. SA application increased the NR activity in all the varieties, but the highest value being recorded from Anjali 26% understress. Moisture stress at PI stage decreased the NR activity to a tune of 42.3% to 49.4% as compared to control depending on the cultivar but application of SA increased the same from 1.2 to 1.7% to the stress treated plants. Under stress condition the NR activity was greatest in Anjali (18.0%) followed by Mandakini (16.8%) whereas the least value was recorded from Parijat (13.9%). Plants subjected to moisture stress at flowering also brought about a decrease in the NR activity in all the varieties to a tune of 35.5 to 40.1% as compared to control (from 23.3 to 11.1 nmol of $NO_3$ reduced g -1 FW of leaf hour -1). Again spraying of salicylic acid to the treated plants increased the NR activity significantly Anjali recorded the highest NR activity having 23.7 nmol of $NO_3$ reduced g -1 FW of leaf per hour whereas least value (17.2 nmol) was noted from Annada.

**Proline Accumulation**

The proline accumulations estimated at different stages of growth of the test varieties have been presented in Table 6. Data presented in the table indicated that irrespective of varieties the proline accumulation increased when the plants are exposed to moisture stress at different growth phases. At tillering stage the stressed plants accumulated more proline compared to control but salicylic acid application to the stress treated plants further enhanced the free proline accumulation to a tune of 7% to 9 % over the stressed plants. Under stressed condition Sahabhagi Dhanaccumulated the highest mean quantity of 172.3 µg g-1 FW of leaf but the lowest value of the same was recorded from Parijat (158.7µg g-1 FW leaf); but application of salicylic acid enhanced the proline accumulation significantly. The interaction effect on proline accumulation between the variety and treatment was significant. Moisture stress at PI stage resulted in an increase in the amount of proline accumulation. The stress treatments applied with SA accumulated 1.2% more proline compared to the stress treatments. Under stressed treatments Anjali accumulated more proline 180.3µg g-1 followed by Mandakini 168.87µg g-1. Similar trend was noticed at floweringstage. The proline accumulation was recorded highest in Anjali 77.3% followed by Mandakini 69.7% under stressed condition but application of SA further increased the proline content to a tune of 22 % and 21 % compared to earlier. The interaction effect on proline content between the variety and the treatment was found significant.

**Table 5.** Effect of moisture stress on Nitrate Reductase (nM $NO_3$ $g^{-1}$ $hr^{-1}$) activity on different growth stages of rice cultivar

| Treatment | Tillering | | | PI stage | | | Flowering stage | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2011 | 2012 | Pooled | 2011 | 2012 | Pooled | 2011 | 2012 | Pooled |
| T1 | 31.9 | 32.5 | 32.33 | 30.42 | 31.54 | 30.9 | 22.96 | 23.56 | 23.26 |
| T | 21.02 | 24.6 | 23.0 | 26.6 | 26.56 | 26.6 | 19.4 | 20 | 19.7 |
| T3 | 22.2 | 25.73 | 24.0 | 27.4 | 28.4 | 27.9 | 20.5 | 20.7 | 20.6 |
| T4 | 32.28 | 32.9 | 32.2 | 15.6 | 16.1 | 15.8 | 18.9 | 11.7 | 15.3 |
| T5 | 32.1 | 33.0 | 32.5 | 15.8 | 16.3 | 16.1 | 21.8 | 11.89 | 16.8 |
| T6 | 31.96 | 32.8 | 32.3 | 30.5 | 31.8 | 31.2 | 14.34 | 7.81 | 11.1 |
| T7 | 31.8 | 33.0 | 32.4 | 30.7 | 31.9 | 31.3 | 19.8 | 10.78 | 15.3 |
| Mean | 29.1 | 30.6 | 29.8 | 25.3 | 26.1 | 25.7 | 19.7 | 15.2 | 17.4 |
| SEm(±) | 0.149 | 0.150 | 0.105 | 0.207 | 0.209 | 0.147 | 0.372 | 0.382 | 0.266 |
| C.D (0.05 | 0.421 | 0.435 | 0.317 | 0.586 | 0.59 | 0.441 | 1.051 | 1.055 | 0.799 |
| *Variety* | | | | | | | | | |
| Mandakini (V1) | 32.07 | 32.4 | 32.23 | 27.45 | 28.1 | 27.8 | 20 | 22.8 | 21 |
| Sahabhagi Dhan (V2) | 28.01 | 28.6 | 28.3 | 24.12 | 24.7 | 24.4 | 18.78 | 21.4 | 20.1 |
| Parijata (V3) | 26.81 | 27.3 | 27.1 | 22.7 | 23.2 | 22.9 | 18.22 | 20.8 | 19.5 |
| Annada (V4) | 25.24 | 25.7 | 25.5 | 23.31 | 23.8 | 23.6 | 19.3 | 22 | 20.6 |
| Anjali (V5) | 33.2 | 33.9 | 33.5 | 28.84 | 29.4 | 29.1 | 22.05 | 25.1 | 23.5 |
| Mean | 29.1 | 29.6 | 29.32 | 25.3 | 25.8 | 25.5 | 19.68 | 22.4 | 21 |
| SEm(±) | 0.126 | 0.14 | 0.09 | 0.245 | 0.251 | 0.175 | 0.189 | 0.19 | 0.166 |
| C.D(0.05) | 0.411 | 0.511 | 0.28 | 0.800 | 0.804 | 0.52 | 0.616 | 0.62 | 0.49 |

**Table 6.** Effect of moisture stress on Proline content (μgg-1) at different growth stages of rice cultivar

| Treatment | Tillering | | | PI stage | | | Flowering stage | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2011 | 2012 | pooled | 2011 | 2012 | pooled | 2011 | 2012 | Pooled |
| T1 | 166.5 | 169.4 | 168.0 | 146.4 | 143.6 | 145.0 | 87.7 | 85.5 | 86.6 |
| T2 | 229.0 | 233.0 | 231.0 | 152.7 | 159.8 | 156.3 | 131.0 | 133.1 | 132.1 |
| T3 | 181.14 | 184.2 | 183.0 | 153.7 | 160.7 | 157.2 | 142.4 | 144.7 | 143.5 |
| T4 | 228.08 | 231.9 | 230.0 | 178.1 | 184.4 | 181.2 | 111.6 | 108.8 | 110.2 |
| T5 | 230.18 | 234.0 | 232.1 | 165.5 | 160.7 | 163.1 | 134.8 | 131.4 | 133.1 |
| T6 | 230.52 | 234.3 | 232.4 | 154.7 | 161.8 | 159.5 | 105.3 | 102.6 | 103.9 |
| T7 | 229.74 | 214.8 | 223.45 | 156.0 | 163.1 | 159.5 | 94.7 | 92.3 | 93.5 |
| Mean | 213.5 | 217.2 | 215.3 | 158.1 | 162.2 | 160.6 | 118.9 | 118.5 | 118.7 |
| SEm(±) | 0.036 | 0.065 | 0.035 | 0.140 | 0.43 | 0.57 | 0.075 | 0.087 | 0.057 |
| C.D (0.05) | 0.103 | 0.34 | 0.107 | 0.396 | 0.54 | 1.51 | 0.212 | 0.43 | 0.171 |
| *Variety* | | | | | | | | | |
| Mandakini (V1) | 214.3 | 218.5 | 216.4 | 157.2 | 153.0 | 155 | 125.9 | 127.9 | 126.9 |
| SahabhagiDhan(V2) | 219.25 | 223.6 | 221.4 | 160 | 164.0 | 162.0 | 117.3 | 119.1 | 118.2 |
| Parijata (V3) | 202.7 | 206.7 | 204.7 | 178.0 | 182.6 | 180.3 | 112.6 | 114.4 | 113.5 |
| Annada (V4) | 220.57 | 225.0 | 222.7 | 160.3 | 177.6 | 168.8 | 125.9 | 127.9 | 126.9 |
| Anjali (V5) | 202.7 | 206.7 | 204.7 | 122.8 | 120.0 | 121.3 | 112.4 | 114.2 | 113.3 |
| Mean | 211.6 | 216.1 | 214.7 | 155.6 | 159.4 | 157.4 | 118.9 | 120.8 | 119.8 |
| SEm(±) | 0.033 | 0.47 | 0.177 | 0.121 | 0.23 | 0.12 | 0.036 | 0.045 | 0.02 |
| C.D (0.05) | 0.108 | 0.21 | 0.533 | 0.393 | 0.56 | 0.37 | 0.117 | 0.223 | 0.085 |

## DISCUSSION

Effect of moisture stress was assessed on the concentration of chlorophyll contents of leaf at different stages of growth of rice plants. The concentration of chlorophyll a/b and total chlorophyll decreased in response to moisture stress irrespective of growth and the cultivars. Decrease in chlorophyll content due to instability of protein and destruction of chlorophyll by increased activity of chlorophyll degrading enzymes and chloropyllase under stress condition. Water deficit reduces the resistance of chloroplast and reduces the chlorophyll synthesis enzyme activity and increased glutamate kinase and abnormal opening and closing of stomata. Similar reduction in chlorophyll content in rice leaves have been observed in previous studies (Ozturk *et al.*, 2005; Sadeghand Nafchi, 2014).

Anjali and Mandakini registered lowest decrease compared to other variety under moisture deficit. Salicylic acid foliar spraying in stressed plant enhanced chlorophyll content in all the varieties to a tune of 8.4 to 18 % over the stressed plant. Perhaps salicylic acid probably prevents from action of chlorophyll oxidase enzymes and increased glutamate ligase activity with development of chloroplast and mechanisms associated relating to chlorophyll synthesis (Jahncke *et al.*, 2008). Regulating stomatal frequency and maintaining the structure due to salicylic acid application could be reasons of increasing of chlorophyll (Kizhedath and Suneetha, 2011). The present results matches with the result of some previous studies (Sayyari *et al.*, 2013; Hossein *et al.*, 2015). The chlorophyll stability index (CSI) exhibited similar decreasing trend with imposition of water deficit. The reduction in CSI ranged from 11.6 % to 35.7 % but application of SA enhanced the CSI. This result corroborates with the results of a previous study (Reddy *et al.*, 2003).

From the present experiment it was revealed that during water deficit the NR activities decreased compared to control irrespective of growth stages and varieties. The reduction was 28%, 48.8 % and 52.2 % at tillering, PI and flowering stage respectively. NR activity is concerned drought at flowering stage was more innocuous than at tillering stage and the present findings agrees with the reports of some previous studies (Saxena *et al.*, 2001; Corria, 2005). Application of salicylic acid increased the NR activity in all stages of growth particularly attillering stage enabling maximum nitrogen absorption. The decrease of NR activity during the moisture stress might be due to decrease in water potential, cell division and cytokinin content in the leaves (Manian *et al.*, 1987); whereas salicylic acid application enhanced the above enzyme activity due to available of $NO_3$ at the enzyme sites.

Drought stress generally produces numerous organic osmolite more specifically, such as proline.

Proline accumulation is considered as a sign of drought injury, the concentration of which builds up under stress due to hydrolysis of protein. Proline in a solution, decreases the osmotic potential, maintains cell swelling, stabilize and protects membrane system, prevents protein breakdown and ultimately reduce the negative effects of stress & thus prevents further water loss in drought stress condition with lesser damage caused by free radicals (Solgi *et al.,* 2009). Proline increased to a tune of 37.5 %, 13.7 % and 19.9 % over the control at tillering, PI & flowering stage respectively. The variety Anjali recorded lower accumulation of proline. Apart from protein hydrolysis. Proline accumulation under stress has been ascribed due to stimulation of synthesis from glutamic acid perhaps due to loss of feedback inhibition of the synthesis of intermediates 4.- pyroline - 5 – carboxylate inhibition of proline oxidation, impaired incorporation of the free proline into protein and reduced export of proline via phloem tissue (Saghfi *et al.,* 2014; Sritharan *et al.,* 2015).

## CONCLUSION

The present study indicated that the response of moisture stress is different at vegetative and reproductive phases. Moisture stress imposed at the reproductive stage of the rice crop hastened senescence and other primary and secondary injuries. From the result of the present experiment, several biochemical traits have been identified. The biochemical parameters such as chlrophyll Stability Index decreased with increase in stress. Among the varieties Anjali was found superior to other varieties in all aspects like prolineaccumulation,NR activity; which are selection criteria for drought resistance. The application of Salicyclic acid on stressed plant played a role in mitigating water deficit injury at critical stages. Notwithstanding the present achievement, further investigation is needed in various agro-climatic conditions of Odisha for continuation vis-a-vis recommendation of best suitable variety to primary stakeholders

## REFERENCES


Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant physiology*. 24(1): 1.

Bates, L. S., Waldren, R. P. and Teare, I. D. 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil*. 39(1): 205-207.

Bohnert, H. J., Nelson, D. E. and Jensen, R. G. 1995. Adaptations to environmental stresses. *The Plant Cell*. 7(7) : 1099.

Chauhan, J. S., Meena, M. L., Saini, M. K., Meena, D. R., Singh, M., Meena, S. S. and Singh, K. H. 2009. Heat stress effects on morpho-physiological characters of Indian mustard (*Brassica juncea* L.). *16th Australian Research Assembly on Brassicas, Ballarat Victoria*. 91-97.

Corría, R. R. 2005. La transmisión y pruebadelderecho a la indemnizaciónpordaño moral. *Vniversitas*. 54(109): 239-259.

Haefele, S. M., Kato, Y. and Singh, S. 2016. Climate ready rice: augmenting drought tolerance with best management practices. *Field Crops Research*. 190 : 60-69.

Hatam, M. I. R. and Hume, D. J. 1976. Relations between nitrate reductase activity and nitrogen accumulation in soybeans. *Canadian Journal of Plant Science*. 56(2): 377-384.

Hossein Khatoonabadi, S., Vasconcelos, N., Bajic, I. V. and Shan, Y. 2015. How many bits does it take for a stimulus to be salient?. In *Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition* (pp. 5501-5510).

Jahncke, J., Saenz, B. L., Abraham, C. L., Rintoul, C., Bradley, R. W. and Sydeman, W. J. 2008. Ecosystem responses to short-term climate variability in the Gulf of the Farallones, California. *Progress in Oceanography*. 77(2-3) : 182-193.

Jimenez, A., Creissen, G., Kular, B., Firmin, J., Robinson, S., Verhoeyen, M. and Mullineaux, P. 2002. Changes in oxidative processes and components of the antioxidant system during tomato fruit ripening. *Planta*. 214(5): 751-758.

Kizhedath, A. and Suneetha, V. 2011. Estimation of chlorophyll content in common household medicinal leaves and their utilization to avail health benefits of chlorophyll. *Journal of Pharmacy Research*. 4(5) : 1412-1413.

Manian, K., Natarajaratnam, N., Ramasamy, P. and Mohanasundaram, K. 1987. Senescence and its role in soybean productivity. *Journal of Agronomy and Crop Science*. 159(3): 202-205.

Mano, N., Mao, F. and Heller, A. 2002. A miniature biofuel cell operating in a physiological buffer. *Journal of the American Chemical Society*. 124(44) : 12962-12963.

Ozturk, M., TurkyilmazUnal, B., García Caparrós, P., Khursheed, A., Gul, A. and Hasanuzzaman, M. 2021. Osmoregulation and its actions during the drought stress in plants. *Physiologia Plantarum*. 172(2) : 1321-1335.

Reddy, T. V. B., Rao, J. P. and Vijayalakshmi, K. 2003. Physiological and biochemical evaluation of groundnut cultivars differing in drought tolerance. *Indian Journal of Plant Physiology*. 8(4): 359-363.

Sadasivam, S. and Manickam, A. 1992. Phenolics. *Biochemical Methods for Agricultural Sciences*. 187-188.

Sadegh-Hassani, F. and Nafchi, A. M. 2014. Preparation and characterization of bionanocomposite films based on potato starch/halloysitenanoclay. *International Journal of Biological Macromolecules*. 67: 458-462.

Saghfi, S. and Eivazi, A. R. 2014. Effects of cold stress on proline and soluble carbohydrates in two chickpea cultivars. *International Journal of Current Microbiology and Applied Sciences*. 3(2): 591-595.

Saxena, D. and Stotzky, G. 2001. Bacillus thuringiensis (Bt) toxin released from root exudates and biomass of Bt corn has no apparent effect on earthworms, nematodes, protozoa, bacteria, and fungi in soil. *Soil Biology and Biochemistry*. 33(9) : 1225-1230.

Sayyari, M., Ghavami, M., Ghanbari, F. and Kordi, S. 2013. Assessment of salicylic acid impacts on growth rate and some physiological parameters of lettuce plants under drought stress conditions. *International Journal of Agriculture and Crop Sciences (IJACS)*. 5(17): 1951-1957.

Solgi, M., Kafi, M., Taghavi, T. S. and Naderi, R. 2009. Essential oils and silver nanoparticles (SNP) as novel agents to extend vase-life of gerbera (*Gerbera jamesonii* cv.'Dune') flowers. *Postharvest Biology and Technology*. 53(3): 155-158.

Sritharan, S., Aaleti, S., Henry, R. S., Liu, K. Y. and Tsai, K. C. 2015. Precast concrete wall with end columns (PreWEC) for earthquake resistant design. *Earthquake Engineering & Structural Dynamics*. 44(12) : 2075-2092.