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Biochemical indicators in upland rice (*Oryza sativa* L.) under physiological drought condition

Bikha Timung and Bhagawan Bharali

Abstract
Rice grown in Assam suffers from intermittent deficit of water in upland condition during Rabi season (January to May). The ailment caused by water stress of rice crop can be judged at subclinical level incorporating some of the biochemical changes in plants at different growth stages of the crop. Nitrogen in leaf is one of the key parameters involved in many physiological processes e.g chlorophyll formation modulating the rate of photosynthesis in plants, and it is restrained by drought stress in crops. SPAD (soil plant analysis development) values measured by chlorophyll meter are the yardstick for judicious application of nitrogen fertilizer for enhancing N status in rice crop in situ. Fixed time nitrogen management (FTNM) is considered as a useful tool practised in rice crop. As per FTNM, 40KgNha^{-1} was applied as basal, and subsequent dose of nitrogen was applied according to the critical need of the crop, determined by the SPAD values against a control without basal N. Thus, the cumulative nitrogen received by the crop in the situation of physiological drought (No water+ 5000ppm of 6000PEG spray at tillering and heading stages against normal irrigation) was 140kgha^{-1}. Five traditional upland genotypes (Mipholong, Balam, Sok Langlu, Sovak and Inglongkiri) were incorporated in the studies, where Inglongkiri emerged as the most physiologically efficient genotype. It was enriched by the sound biochemical features viz., higher leaf N(1.7-2.5% %), NR activity (480-501 µmoleNO_2^- g^{-1} f.w. hr^{-1}), proline content (261-326 µgmol^{-1}g^{-1} f.w.), total carbohydrate content (9.8-11.3mg g^{-1} d.w), P^n rate (10.8-12.4µmolesCO_2 absorbed m^{-2}sec^{-1}) envisaged by the higher SPAD values (32 to 32) irrespective of drought and unstressed plants.

Keywords: Upland rice, nitrogen, NR, proline, carbohydrate, SPAD, FTNM

Introduction

Rice (*Oryzae sativa* L.) is grown in sub-tropics at congenial temperature (21° C to 31° C) and pH (5.5 to 7.0) but, soil water deficit impairs the plant growth and development, and limits yield significantly (Chandrasekaran *et al*., 2007) [5]. Plant's reaction to drought is recognised in terms of physiological and metabolic events (Hansen, 1980; Timung *et al*., 2017) [17, 6]. The upland rice (*Ahu)* is the second major group of rice in Assam, which is used as buffer stock for food grain and fodder during lean period. The lower productivity of upland *Ahu* rice is due to low soil moisture during germination, intermittent moisture stress during panicle initiation. The upland topography and soil type vary in Assam. The higher elevation and the uneven topography make it impossible to retain water for a longer period of time. Soils are generally sandy-loam with poor native fertility and low moisture retention capacity. Low water retention due to percolation causes moisture stress soon after rain stops. Therefore, moisture stress is one of the main causes of lower productivity of upland rice in Assam, which is only 0.9 t ha^{-1} as compared to the national average of about 1.9 t ha^{-1} (Torenpi and Bharali, 2019) [47].

Nitrogen plays a pivotal role in chlorophyll synthesis for photo assimilation leading to plant growth and development (Lenka *et al*., 2009) [32]. The supply of useable nitrogen and the rate of its losses from the soil affect the sustainability in production. If mismanaged, it can result in economic loss to the producer and have environmental repercussions, or both. However, nitrogen is used effectively when there is availability of moisture. Instead, the nitrogenous fertilizer enhances water productivity (Pandey *et al*., 2001) [36]. Specific Nutrient management (SSNM) especially Fixed time nutrient management (FTNM) is followed to increase physiological efficiency and productivity of rice crop (Peng *et al*., 1996; Dobermann *et al*., 2002) [39, 11]. In Assam, implication of FTNM to excel the biochemical markers in upland rice from Karbi Anglong district of Assam under physiological drought situation has not been accomplished so far. Therefore, the current manuscript discusses several such indicators for judging the efficiency of some upland rice genotypes grown under physiological drought situation.

Materials and Methods

A pot experiment laid in Randomised Block Design, replicated thrice, was carried out with five upland (Ahu) rice genotypes (viz., Mipholong, Balam, Sok Langlu, Sovak and Inglongkiri) in the department of Crop Physiology, Assam Agricultural University, Jorhat which is geographically situated at 87 meter above the mean sea level, $26^{0}45'$N latitude and $94^{0}12'$E longitude. The climatic condition of Jorhat as a whole, is subtropical, humid, dry summer and cold winter. The total rainfall for the crop season was 1314.6 mm.

In the pot culture, seedlings (n=3) were raised from direct sowing of seeds in the earthen pots. The whole amount of P&K @ 20:20 in the form of SSP (Single Super Phosphate) and MoP (Muriet of Potash) were applied as basal. N was applied based on Fixed Time Nitrogen Management (FTNM) method, where, the recommended dose of nitrogen (@40Kgha^{-1}) was used as base nitrogen against a control (0 kg Nha^{-1}) for all the genotypes under two regimes of water (irrigated & physiological drought), and subsequent doses of nitrogen were applied in splits demanded by the crop determined using SPAD values, measured by Chlorophyll meter.

The physiological drought was imposed to the potted plants inside a poly house to protect from the natural precipitation. In one set, rice genotypes were grown with full irrigation while in the other set, the same genotypes were provided with two cycles of seven days drought viz., at 30 days after sowing (during tillering stage), and another at 90 days after sowing (heading stage) by suspending irrigation, and further misting with PEG 6000 (5000ppm ≈ -0.05bars) uniformly, for inducing physiological drought in plants (Chutia and Borah, 2012) [9]. Then, the pots were kept in nearby field outside the poly house during the rest of the growth period.

The rate of Photosynthesis in leaves was determined using Infra Red Gas Analyzer (IRGA: Model LI-COR 6400). Fully expanded upper most leaf was used for measuring the gas exchange parameters. The leaves were illuminated on a clear sunny days between 10 AM to 12 Noon, when photosynthetic active radiation (PAR) ranged between 900-1500 µE m^{-2}s^{-1}. *In vivo* nitrate reductase activity was assayed following the method of Keeper *et al.* (1971) [26]. Free proline was estimated in leaves by using formula described by Bates *et al.* (1973) [1].

In the experiment, five randomly selected leaves were ground in electrical grinder after drying in oven at 80°C into a constant weight. All the powdered materials were sieved and stored in desiccators separately for use in the laboratory estimation of Nitrogen and total carbohydrates in leaf. Total carbohydrate contents in leaf tissues were estimated following Anthrone method (Hedge *et.al*. 1962[16]). The modified Kjeldhal method (Jackson, 1973) [23] was used to determine nitrogen content, which is based on catalytic conversion of organic nitrogen into ammonia and its subsequent estimation by acid base titration**.**

Data for each parameter was analysed by Fisher's method of analysis of variance (Panse and Sukhatme, 1978) [38]. Significance or non-significance of variance due to the treatment effects was determined by calculating the respective 'F' values. The standard error of the means (S.E.diff.) was calculated using error mean square and pooled number of replication, where the critical difference (CD) was calculated to test the significance of treatment or genotypes at P(0.05) probability level.

Results and Discussion

In the investigation, the crop was subjected to water stress by withholding irrigation for seven days plus spraying with PEG-6000 (5000 ppm) both at maximum tillering and heading stages. The crop was deprived of natural precipitation during these periods inside a poly house except supply of live saving water while soil Tensiometer auto-fixed its readings at 80 centibars, and crop wilted visually. All the genotypes received a range of temperature (20-35^{0} C), Relative humidity (32-83%) and light intensity (1200-1600 Lux) during the drought treatments in the months of April and May. The crop genotypes were kept outside the poly house during the rest of the growth period, where the plant experienced similar situation in the open filed. As such, physiological drought imposed at the certain periods remained only a variable abiotic factor to bring physiological changes in the upland rice varieties.

In the study, SPAD values differed significantly at maximum tillering and heading stages of the crop due to the imposed physiological drought (Table 1). The highest SPAD value was recorded in Inglongkiri (32.46) followed by Sovak (30.75), and the lowest was in Balam (26.753) < Sok langlu (28.723) at maximum tillering stage under irrigated condition. Inglongkiri (31.837) indicated the highest SPAD value >Sovak (29.85), and the lowest SPAD value was marked in Balam (26.753) < Sok langlu (27.877) under drought condition at the same stage. The highest SPAD value was found in Inglongkiri (34.623) > Sovak (32.7983), and the lowest was in Balam (28.723)< Sok langlu (29.82). Inglongkiri (33.83) displayed the highest SPAD value > Sovak (31.513) whereas Balam (27.833) < Sok langlu (28.853) had the least values under drought condition at heading stage under irrigated condition. So, SPAD readings were significantly altered by the stress at maximum tillering and heading stages.

Table 1: Soil plant analysis development (SPAD) values in leaf at different growth stages of rice crop under water regimes

SPAD values at maximum tillering stage				SPAD values at heading stage		
Treatments→ Genotypes↓	**Irrigated**	**Drought**	**Mean**	**Irrigated**	**Drought**	**Mean**
Mipholong	29.863	28.843	29.353	30.833	29.123	29.978
Balam	26.753	25.977	26.365	28.723	27.833	28.278
Sok Langlu	28.723	27.877	28.30	29.820	28.853	29.337
Sovak	30.750	29.85	30.30	32.793	31.513	32.153
Inglongkiri	32.460	31.837	32.148	34.623	33.83	34.227
Mean	29.71	28.877		31.359	30.231	
	S.E.diff (±)	C.D. (0.05)		S.E.diff (±)	C.D. (0.05)	
Treatment (T)	0.019	0.04		0.036	0.076	
Genotype (G)	0.03	0.063		0.057	0.12	
T X G	0.042	0.089		0.08	0.17	

Moisture stress reduced SPAD values in all genotypes of rice in comparison to irrigated one. Drought reduced SPAD values by 2.804% and 3.598 % at maximum tillering and heading stages respectively (Fig.1). At maximum tillering stage, the highest reduction of SPAD was found in varieties Mipholong (3.41 %) > Sok langlu (2.94 %) and Sovak (2.92 %). Similarly, at heading stage, the lowest reduction of SPAD was found in Inglongkiri (2.29%) < Balam (3.09 %) and Sok langlu (3.24%). The differences in reductions of SPAD values is genotype specific, which has a background of FTNM for N requirement at the crop growth periods (Xu *et al.* (2000)) [48]; Talwar *et al.* (2011) [46], and Sudhakar *et al.*, (2006) [44]. One advantage is that measurement of SPAD values is non-destructive for assessing the status and application of N in the crop.

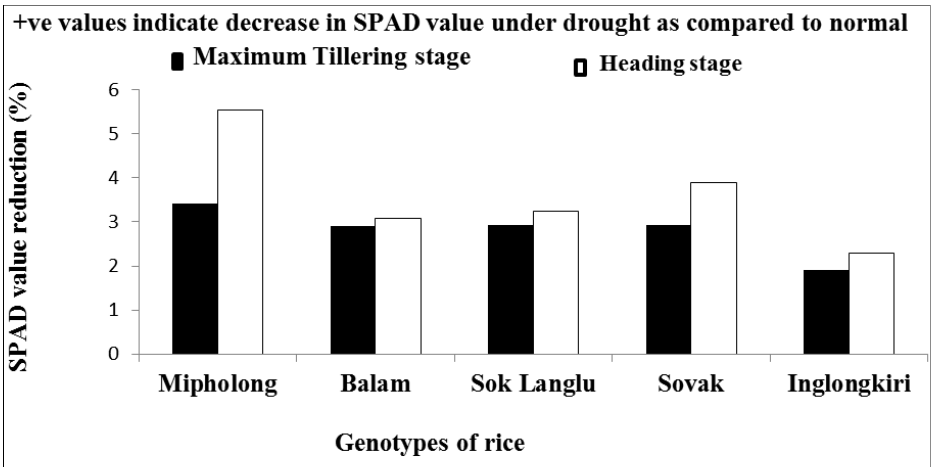

Fig 1: Changes of SPAD values under drought as compared to irrigation

Nitrogen content in leaf tissues at maximum tillering and heading stages was altered significantly by the physiological drought (Table 2.**).** The variety, Inglongkiri (1.817%) >Sovak (1.423 %) recorded the highest, and Balam (1.163 %) < Mipholong (1.277%) had the lowest N contents under irrigated condition. Under drought condition, the highest & lowest N contents was found in Inglongkiri (1.533 %)> Balam (0.987%). As regard to leaf nitrogen content at heading stage, the highest value was recorded in Inglongkiri (2.76%) > Sovak (2.12%), and the lowest was present in Balam (1.333 %) < Mipholong (1.477%) under irrigated condition. Under drought condition also Inglongkiri (2.147%) maintained the highest nitrogen status followed by Sovak (1.9 %). The lowest leaf nitrogen content was recorded in Balam (1.12%) < Mipholong (1.377 %). It was interesting to record that there was gradual increase in leaf nitrogen content from maximum tillering to heading stage under both irrigated and drought conditions.

Table 2: Leaf nitrogen (N) content at different stages of rice crop under water regimes

N content at maximum tillering stage (%)				**N content at heading stage (%)**		
Treatments→ genotypes↓	**Irrigated**	**Drought**	**Mean**	**Irrigated**	**Drought**	**Mean**
Mipholong	1.277	1.107	1.192	1.477	1.377	1.427
Balam	1.163	0.987	1.075	1.333	1.120	1.227
Sok Langlu	1.347	1.13	1.238	1.870	1.453	1.662
Sovak	1.423	1.317	1.370	2.12	1.90	2.01
Inglongkiri	1.817	1.533	1.675	2.76	2.147	2.453
Mean	1.405	1.215		1.912	1.599	
	S.E.diff (±)	C.D. (0.05)		S.E.diff(±)	C.D. (0.05)	
Treatment (T)	0.013	0.028		0.009	0.019	
Genotype (G)	0.021	0.044		0.014	0.03	
T X G	0.029	0.062		0.02	0.042	

Moreover, physiological drought decreased the N accumulation in all genotypes of rice (Fig.2). The highest reduction was observed in Balam (91.51%) at maximum tillering, and in Sok langlu (22.29 %) at heading stage, whereas the lowest reduction was in Sovak (7.44 %) at maximum tillering and Mipholong (6.77 %) at heading stage. Thus, Balam contained the highest amount of leaf nitrogen at reproductive stage under drought as compared to irrigated. condition This indicates better ability of the genotype in acquiring N either from soil or applied nitrogen, and in remobilizing N under favourable water regime (normal) condition (Ghanbari *et al*., 2013, Nakayama *et al*., 2007) [15, 34]. According to Janadhan and Murty (1980) [24] nitrogen present in all plant parts decreases commensuration with the progressive growth stages of the crop.

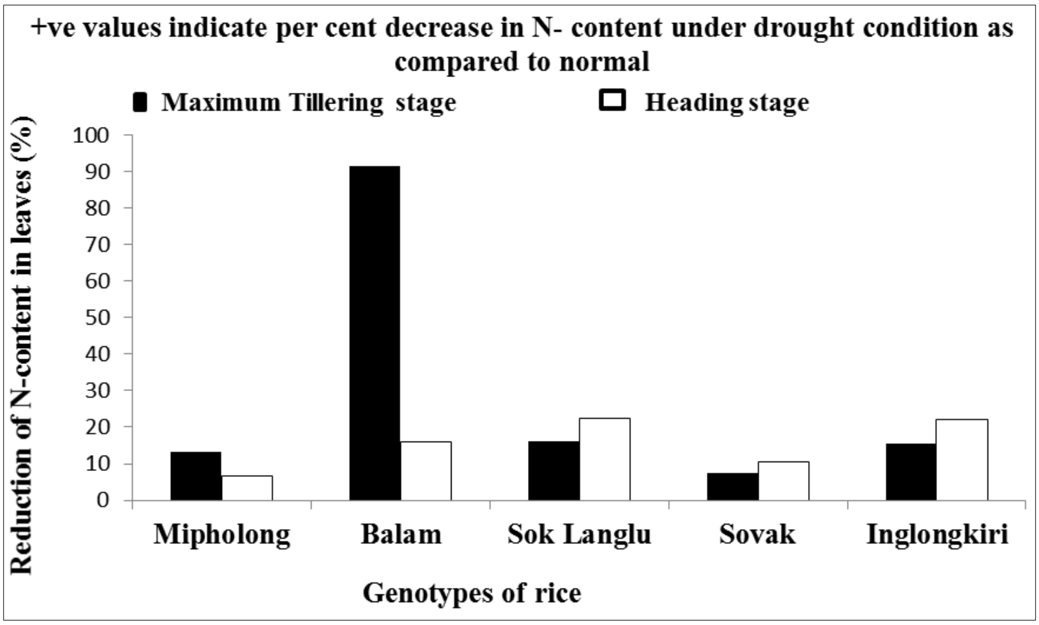

Fig 2: Changes of N-content of leaf tissue under drought as compared to irrigation

Physiological drought brought significant changes in Proline contents in leaf tissues at maximum tillering and heading stages of the crop (Table 3). Inglongkiri (215.267 µgmol^{-1}g^{-1}f.w) followed by (>) Sok langlu (213.667 µgmol^{-1}g^{-1}f.w), accumulated the highest proline contents in leaf, whereas the lowest proline content was recorded in Balam (208.833 (µgmol^{-1}g^{-1}f.w) < Mipholong (212.5 µgmol^{-1}g^{-1}f.w) under irrigated condition. Under physiological drought, Inglongkiri (307.5 µgmol^{-1}g^{-1}f.w) synthesised the highest proline, and Sok langlu (293.667 µgmol^{-1}g^{-1}f.w) was at the lowest end under physiological drought. At heading stage, under irrigated condition, Inglongkiri (299.533 µgmol^{-1}g^{-1}f.w) showed the highest proline accumulation > by Sok langlu (299.167 µgmol^{-1}g^{-1}f.w), and lowest proline accumulation was found in Balam (284.533 µgmol^{-1}g^{-1}f.w) < Mipholong (297.533 µgmol^{-1}g^{-1}f.w). Under physiological drought, Inglongkiri (354.033 µgmol^{-1}g^{-1}f.w) topped the rank of proline accumulation, and Sok langlu (333.667 µgmol^{-1}g^{-1}f.w) had the lowest proline accumulation in leaf. In fact, proline accumulation is the symptom or positive consequence of reduced water status in plants. There is a relationship between proline accumulation in leaves tissue and the moistures content in soil. It has been speculated that proline could represent an acclimatisation mechanism for plant survival during a period of drought stress (Clifford *et al*., 1998; Lakmini *et al*., 2006) [10, 29]. Our studies confirm, too, significant increases in proline contents with its variation in the rice genotypes subjected to physiological drought condition (Fig.3).

Table 3: Proline content in leaves at different growth stages of rice crop under water regimes

Proline content in leaves (µgmol^{-1}g^{-1} f.w.) at maximum tillering stage				Proline content (µgmol^{-1}g^{-1} f.w.) at heading stage		
Treatments→ Genotypes↓	Irrigated	Drought	Mean	Irrigated	Drought	Mean
Mipholong	212.50	309.433	260.967	297.533	348.833	323.18
Balam	208.833	306.267	257.55	284.533	346.70	315.61
Sok Langlu	213.667	293.667	253.667	299.167	333.667	316.41
Sovak	212.733	299.40	256.067	298.733	338.633	318.68
Inglongkiri	215.267	307.50	261.383	299.533	354.033	326.78
Mean	212.60	303.253		295.90	344.373	
	S.E.diff(±)	C.D.(0.05)		S.E.diff (±)	C.D. (0.05)	
Treatment (T)	0.133	0.281		0.129	0.273	
Genotype (G)	0.21	0.444		0.204	0.431	
T X G	0.297	0.628		0.288	0.61	f.w.: fresh weight

The genotype Balam (46.65 %) and Sok langlu (37.44 %) experienced the highest and lowest increments in proline contents in plants at maximum tillering stage respectively. At heading stage, the highest and lowest proline accumulation was also maintained by the same varieties viz., Balam (21.84 %) and Sok langlu (11.53 %) accordingly. The stress was created by withholding water for seven days, and simultaneous spraying the plants with PEG-6000 (5000ppm). In general, Polyethylene glycol (PEG) is preferred over Mannitol as an external osmoticum for experiments on water relations (Hohl and Peter, 1991) [22]. Proline accumulation in rice may be controlled genetically (Pandey and Agarwal, 1998) [37]. Under water stress, accumulated proline might act as a compatible solute regulating and reducing water loss from the plant cell during water deficit (Yokota *et al*., 2006) [26]. It plays an important role in osmosis balance (Fedina *et al*., 2002) [13]. Proline accumulates under stress also supplies energy for survivor and growth, and thereby helps the plants to tolerate stress condition (Kumar *et al*., 2011) [27]. Thus, the proline content is a good indicator for screening tolerance of genotypes in water scarcity related stress condition (Bayoumi *et al*., 2008; Rahdari *et al*., 2012) [2, 41]. In here, on an average, the cultivar Balam accumulated 19.956% more proline in water stressed leaf tissues than other genotypes, and emerges as drought tolertanty. On the other hand, Sovak might be susceptible to the same stress situation as it accumulated

6.26% lesser amount of proline under water stress condition. Similar results on proline accumulation under drought were also reported elsewhere in the past (Ram *et.al.* 1988; Narayan and Anuradha, 1991; Kohl *et.al*. 1991) [42, 35, 27].

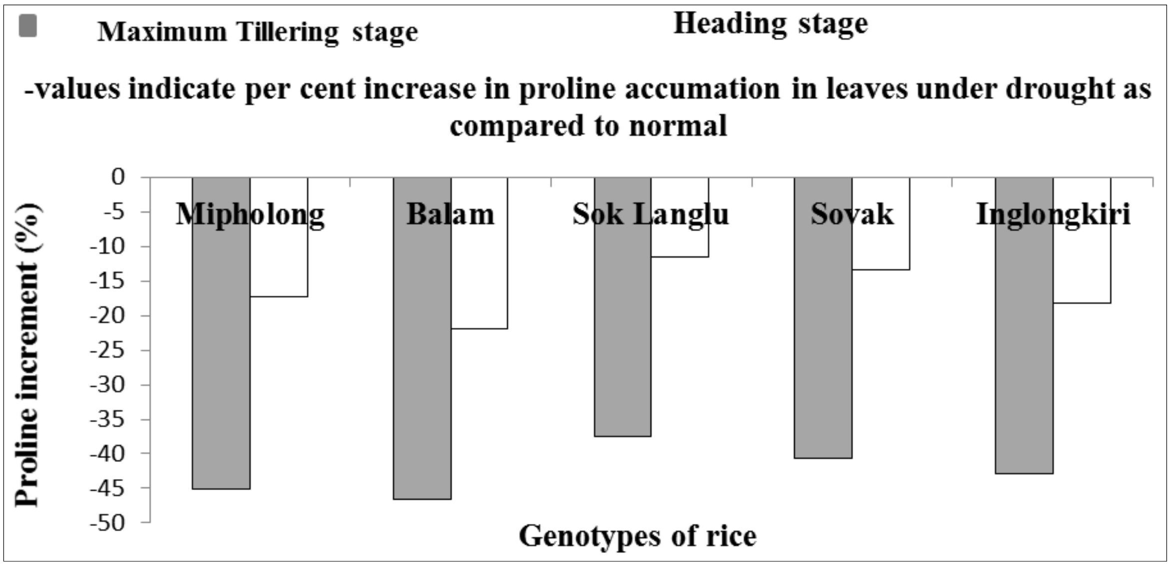

Fig 3: Changes of proline under drought as compared to irrigation

Nitrate Reductase (NR) activity in leaf varied significantly due to physiological drought at maximum tillering and heading stages of the crop (Table 4). At maximum tillering stage, the highest NR activity was recorded in Inglongkiri (487.667µmoleNO$_2^-$g^{-1}f.w. hr^{-1}) followed by (>) Sok langlu (457.157µmoleNO$_2^-$g^{-1}f.w. hr^{-1}), and the lowest was recorded in Balam (387.307µmoleNO$_2^-$g^{-1}f.w. hr^{-1}) < Mipholong (395.177µmoleNO$_2^-$g^{-1}f.w. hr^{-1}) under irrigated condition. Under drought condition also, Inglongkiri (472.29µmoleNO$_2^-$g^{-1}f.w. hr^{-1}) maintained the highest NR activity followed by Sok langlu (447.56µmoleNO$_2^-$g^{-1}f.w. hr^{-1}), whereas and the lowest NR activity was recorded in Balam (376.467µmoleNO$_2^-$g^{-1}f.w. hr^{-1}) < Mipholong (383.363(µmoleNO$_2^-$g^{-1}f.w. hr^{-1}). At heading stage, Inglongkiri (513µmoleNO$_2^-$g^{-1}f.w. hr^{-1}) showed the highest NR values > Sok langlu (487.763 µmoleNO$_2^-$g^{-1}f.w. hr^{-1}), and the lowest was recorded in Balam (396.197 µmoleNO$_2^-$g^{-1}f.w. hr^{-1}) < Mipholong (412.247 µmoleNO$_2^-$g^{-1}f.w. hr^{-1}) under irrigated growth condition. Under drought condition also Inglongkiri (487.87 µmoleNO$_2^-$g^{-1}f.w. hr^{-1}) maintained the highest NR activity > Sok langlu (478.717 µmoleNO$_2^-$g^{-1}f.w. hr^{-1}), and the lowest was also recorded in Balam (382.63 µmoleNO$_2^-$g^{-1}f.w. hr^{-1}) < Mipholong (391.263 µmoleNO$_2^-$g^{-1}f.w. hr^{-1}). However, there were significant reductions in Nitrate Reductase (NR) activity in leaf tissues under physiological drought at both maximum tillering and heading stages of the crop **(**Fig. 4**)**.

Table 4: Nitrate reductase (NR) at different growth stages of rice crop under water regimes

NR at maximum tillering stage (µmoleNO$_2^-$g^{-1}f.w. hr^{-1})				NR at heading stages (µmoleNO$_2^-$ g^{-1} f.w. hr^{-1})		
Treatment→ Genotypes↓	**Irrigated**	**Drought**	**Mean**	**Irrigated**	**Drought**	**Mean**
Mipholong	395.177	383.363	389.27	412.247	391.263	401.755
Balam	387.307	376.467	381.887	396.197	382.63	389.413
Sok Langlu	457.157	447.56	452.358	487.763	478.717	483.24
Sovak	436.317	422.047	429.182	451.61	445.283	448.447
Inglongkiri	487.667	472.29	479.978	513.523	487.87	500.697
Mean	432.725	420.345		452.268	437.153	
	S.E.diff (±)	C.D.(0.05)		S.E.diff(±)	C.D.(0.05)	
Treatment (T)	0.429	0.908		0.258	0.547	
Genotype (G)	0.678	1.436		0.408	0.864	f.w.: fresh weight
T X G	0.959	2.031		0.577	1.223	

Among the genotypes, Mipholong had the highest reduction in NR activity (5.09 %) > Inglongkiri (4.99 %) under drought as compared to irrigated condition. The lowest reduction in NR was shown in Sovak (1.4 %) under drought as compared to normal. On an average, at heading stage, the highest NR reduction was found in Sovak (3.27%) > Inglongkiri (3.15 %), while the lowest reduction was found in Sok langlu (2.09 %). Overall, NR reduction was more at heading (3.34%) stage than at maximum tillering (2.86%) stage of the genotypes. Lower activity of NR in leaf tissues of plant is as an indication of the poor efficiency of genotypes for nitrogen assimilation (Sarkar *et al*. 1991) [43].

There are evidences that Polyethylene glycol induces physiological stress, results in free amino acids and lowers NR activity in pearl millet (Hanson *et.al.*, 1981; Hanson *et al*., 1982) [9, 8]. Water stress also lessens maximal extractable foliar NR activity in plants (Plaut, 1974; Heuer *et al*., 1979; Talouizite and Champigny, 1988; Larsson *et al*., 1989) [40, 20, 45, 39]. In the present investigation, there is reduction of NR activity due to moisture stress, and it is further confirmed by the findings of Ferrario-Mery *et al.*, (1998) [14].

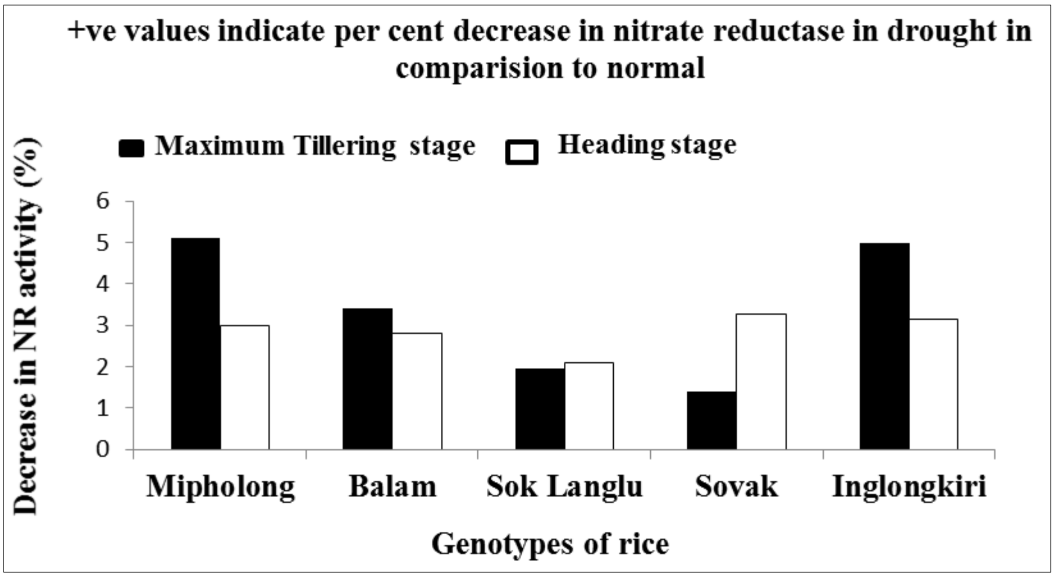

Fig 4: Changes of nitrate reductase under drought as compared to irrigation

Total carbohydrate contents (TCC) in leaf of the genotypes at both maximum tillering and heading stages except inglongkiri varied significantly due to physiological drought (Table 5). At maximum tillering stage, the highest TCC was obtained in Inglongkiri (9.207mgg^{-1}d.w.) followed by (>) Sovak (8.96 mg g^{-1} d.w.), and the lowest TCC was obtained in Balam (5.863mgg^{-1}d.w.) < Mipholong (6.883mgg^{-1}d.w.) under irrigated condition. Inglongkiri (10.30mgg^{-1}d.w.) sustained the highest TCC followed by Sovak (8.113mgg^{-1}d.w.), whereas the lowest TCC was also obtained in Balam (5.153 mgg^{-1}d.w.) < Mipholong (6.107 mgg^{-1}d.w.). Over all, there was increasing trend in TCC from maximum tillering to heading stage of the crop. At heading stage, Inglongkiri (10.147 & 12.403 mgg^{-1}d.w.) preserved the highest TCC followed by Sovak (9.953 & 8.853 mgg^{-1}d.w.) under irrigated and drought conditions respectively. Among the varieties, the varieties Balam (6.883 & 6.15 mgg^{-1}d.w.), and Mipholong (7.92 & 6.71 mgg^{-1}d.w.) possessed the lowest TCC under both irrigated and drought conditions among respectively.

There was a significant negative ramification of moisture stress on TCC in leaves of rice crop except Inglongkiri. This genotype showed increase in TCC under drought in comparison with irrigated condition at both maximum tillering and heading stages. This might be due to the genetic potential for improving the total carbohydrate under the water stress situation. It is apparent from the Fig.5**.** that TCC in leaves was more in heading stage than in maximum tillering stage.

Table 5: Total carbohydrate content (TCC) in leaf at different growth stages of rice crop under water regimes

TCC at maximum tillering stage (mg g^{-1} d.w.)				**TCC at heading stages (mg g^{-1} d.w.)**		
Traetments→ Genotypes↓	**Irrigated**	**Drought**	**Mean**	**Irrigated**	**Drought**	**Mean**
Mipholong	6.883	6.107	6.495	7.92	6.71	7.315
Balam	5.863	5.153	5.508	6.883	6.153	6.518
Sok Langlu	7.857	7.11	7.483	8.883	7.983	8.433
Sovak	8.96	8.113	8.537	9.953	8.853	9.403
Inglongkiri	9.207	10.30	9.753	10.147	12.403	11.275
Mean	7.81	7.138		8.99	7.969	
	SE.diff(±)	C.D.(0.05)		S.E.diff.(±)	C.D.(0.05)	
Treatment (T)	0.014	0.03		0.034	0.072	
Genoype (G)	0.022	0.047		0.054	0.114	d.w.: dry weight
T X G	0.031	0.066		0.076	0.161	

There was remarkable decrease in carbohydrate contents in Balam (12.1%) at maximum tillering stage followed by Mipholong (11.27 %) under drought as compared to irrigated one. Instead, at heading stage, Inglongkiri (22.23%) had the highest increase in TCC. It indicates that this genotype could carry out the process of photosynthesis well, particularly at maximum tillering stage under physiological drought stress. These findings are in accordance with the findings of Dubey and Singh (1999) [12]. Nakayama, (1974) [33] opined that higher level of total carbohydrate plays a protective role against the photo assimilate shortage during stress. It acts as osmolyte under water stress by increasing the gradient for water flux into the cell for maintaining turgor (Bharali *et al*., 2016) [4].

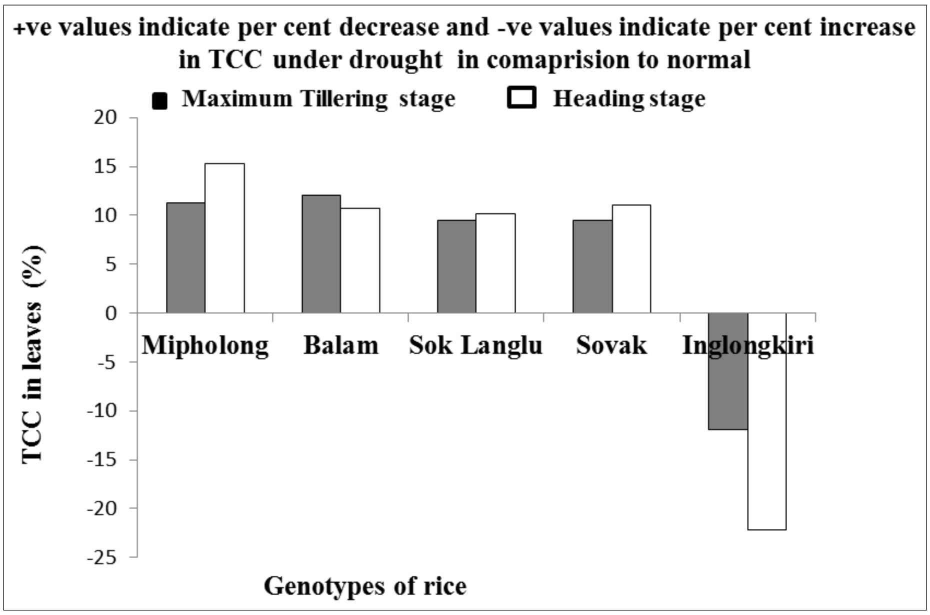

Fig 5: Changes in total carbohydrate content (TCC) under drought as compared to irrigated condition

Physiological drought depressed net photosynthesis (P^n) significantly irrespective of the varieties significantly at different growth stages of the crop **(**Table 6**)**. The P^n rate amplified at heading stage as compared to maximum tillering stage. The highest P^n rate (µmoles CO_2 absorbed $m^{-2}sec^{-1}$) was recorded in Inglongkiri (11.31 µmoles CO_2 absorbed $m^{-2}sec^{-1}$) followed by (>) sovak (8.53 µmoles CO_2 absorbed $m^{-2}sec^{-1}$), and the lowest was recorded in Balam (5.623 µmoles CO_2 absorbed $m^{-2}sec^{-1}$) < Sok langlu (7.531 µmoles CO_2 absorbed $m^{-2}sec^{-1}$) under irrigated condition. Inglongkiri (10.147 µmoles CO_2 absorbed $m^{-2}sec^{-1}$) upheld the highest P^n rate followed by Sovak (7.953 µmoles CO_2 absorbed $m^{-2}sec^{-1}$), and Balam had the least P^n rate (5.573 µmoles CO_2 absorbed $m^{-2}sec^{-1}$) < Sok langlu (7.037 µmoles CO_2 absorbed $m^{-2}sec^{-1}$) under drought condition. At heading stage under irrigated condition, the highest P^n rate (µmoles CO_2 absorbed $m^{-2}sec^{-1}$) was found in Inlongkiri (12.907) > Sovak (9.83 µmoles CO_2 absorbed $m^{-2}sec^{-1}$), and the lowest was in Balam (6.623 µmoles CO_2 absorbed $m^{-2}sec^{-1}$) < Sok langlu (8.813 µmoles CO_2 absorbed $m^{-2}sec^{-1}$). Inglongkiri (11.83 µmoles CO_2 absorbed $m^{-2}sec^{-1}$) possessed the highest P^n rate > Sovak (8.98 µmoles CO_2 absorbed $m^{-2}sec^{-1}$), and the least was in Balam (5.863 µmoles CO_2 absorbed $m^{-2}sec^{-1}$) < Mipholong (7.95 µmoles CO_2 absorbed $m^{-2}sec^{-1}$) under drought condition. In the current study, the rates of net photosynthetic (P^n) in rice at maximum tillering stage was lower than P^n at heading stage under both irrigated and drought conditions. Photosynthesis is the main metabolic process determining carbohydrate formation and crop production, and is eventually all affected by drought stress (Ji *et al.*, 2012; Lauteri *et al.*, 2014; Yang *et al.*, 2014; Bharali and Chack, 2018) [25, 31, 49, 3].

Table 6: Net Photosynthetic rates (P^n) at different growth stages of rice crop under water regime

P^n rate at maximum tillering stage (µmoles CO_2 absorbed $m^{-2}sec^{-1}$)				P^n rate at heading stage (µmoles CO_2 absorbed $m^{-2}sec^{-1}$)		
Treatments→ Genotypes ↓	Irrigated	Drought	Mean	Irrigated	Drought	Mean
Mipholong	7.92	6.71	7.315	8.92	7.95	8.435
Balam	5.623	5.523	5.573	6.623	5.863	6.243
Sok Langlu	7.513	7.037	7.275	8.813	8.113	8.463
Sovak	8.53	7.953	8.242	9.83	8.98	9.405
Inglongkiri	11.31	10.147	10.728	12.907	11.83	12.36
Mean	8.179	7.393		9.419	8.547	
	S.E.diff (±)	C.D. (0.05)		S.E.diff (±)	C.D. (0.05)	
Treatment (T)	0.039	0.082		0.026	0.054	
Genotype (G)	0.061	0.129		0.041	0.086	
T X G	0.086	0.183		0.057	0.122	

The highest reduction in photosynthetic rate was observed in Balam (11.47%) at maximum tillering stage, while at heading stage the highest per cent reduction was found in Mipholong (15.27 %) under drought as compared to normal. The lowest per cent reduction was in Sok langlu (7.94 %) at maximum tillering, and in Balam (0 %) at heading under drought as compared to normal (Fig.6**)**. The major components limiting photosynthesis are the slow diffusion of CO_2 due to early stomatal closure, decreased photochemical efficiency of PS-II and reduced activity of photosynthetic enzymes related to triose-phosphate formation. Change in any of these components alters the final photosynthesis rate. Stomatal and mesophyll conductance to CO_2 often decrease in response to drought. Thus, the ability of a genotype to maintain smooth conductance or gaseous exchange under water-deficits, determines the drought tolerance of the crop (Lauteri *et al.*, 2014) [31]. The increase in leaf photosynthetic rate is important to augment the yield potential of rice (Hirasawa *et al.*, 2010 [21]), because the photosynthetic rate of individual leaf which form the canopy, affects dry matter production via photosynthesis within the canopy.

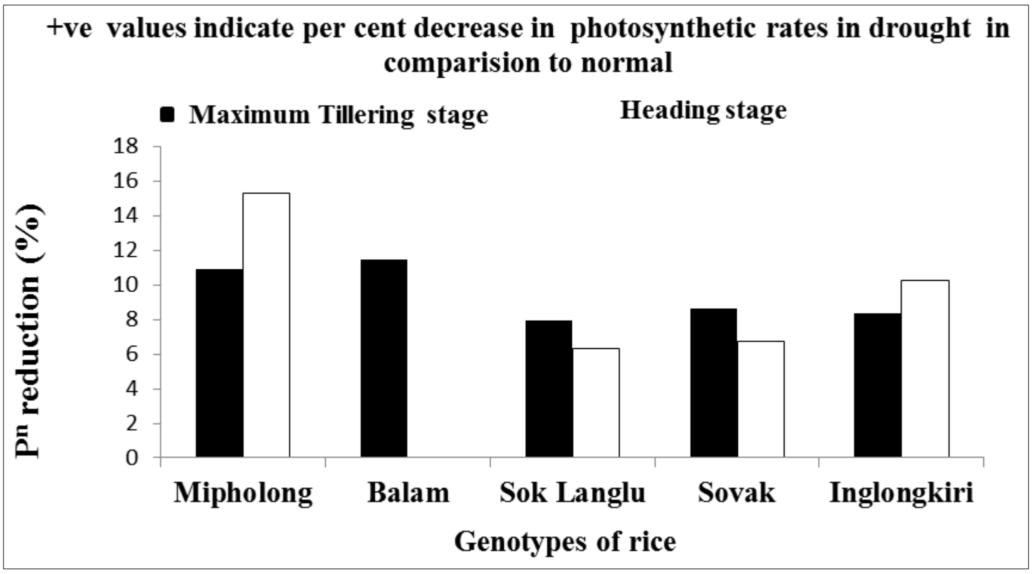

Fig 6: Changes of net photosynthesis (P^n) rates under drought as compared to irrigation

Conclusion

The investigation at subclinical level revealed that Inglongkiri emerged as the most proficient among the tested genotypes under simulated physiological drought condition. This is plausibly supported by the biochemical traits viz., increased quantity of proline to acclimatize, higher rate of photosynthesis, increased NR activity, more nitrogen and carbohydrate contents in leaf for further utilization in grain development under physiological drought stress situation. Further, the SPAD values measured at different growth stages following the FTNM approach, was prolific for applying nitrogen to crop which was found to be equal (140$kgha^{-1}$) for all the varieties irrespective of water regimes, but all other biochemical benchmark differed appreciably in the genotypes under two water regimes.

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