

CHAPTER 3

ROLE OF SILICON FOR DROUGHT RESISTANCE IN UPLAND RICE

3.1 Introduction

Water stress occurs as a result of water deficit or drought. Drought is a meteorological term and can be defined as a period without significant rainfall (Plaut, 1995). Linsley *et al.* (1959) defined drought as a sustained period of time without significant rainfall. While water stress is usually the result of water withholding by human being, however, the terms of water stress and drought are often used interchangeably. The ultimate result of this stress is evaporative dehydration due to water loss at a rate that exceeds the rate of uptake (Plaut, 1995). For a period of dry weather to affect a plant community, the rainfall deficit must lead to a soil water deficit and ultimately to a plant water deficit. The degree to which a rainfall deficit is translated into a soil water deficit depends on the rate of evaporation during the rain-free period, and on the physical and chemical characteristics of the soil. The degree to which a particular soil water deficit influences the plant again depends on the degree of aridity of atmosphere. However, it also depends on a number of plant characteristics that influence water uptake by the plant, the rate of transpiration and the response of the plant to the water deficit so generated. It is the degree to which the plant can withstand the rainfall deficit that constitutes to its drought resistance (Jones *et al.*, 1981).

It has been the common experience of many rice breeders that when rice populations are subjected to drying soil conditions, genotypes are very clearly separated into those that wilt and dry readily and those that maintain a measure of turgor and viability as stress continues. Furthermore, genotypes differ in their recoveries upon rehydration, and the level of genotypic recovery is closely related

(other factors held constant) to its hydration status prior to recovery. Drought resistance in terms of these responses is most likely dependent mainly on one or more of the following components: (i) moderate water-use through reduced leaf area and shorter growth duration, (ii) the ability of the roots to exploit deep soil moisture to provide for evapotranspirational demand, (iii) the capacity for osmotic adjustment which allows it to retain turgor and protect meristems from extreme desiccation, and (iv) the control over nonstomatal water loss from leaves (Nguyen *et al.*, 1997).

For osmotic adjustment, it can counteract the effects of a rapid decline in leaf water potential (Fukai and Cooper, 1996). It is increasingly recognized in several crop plants as an effective component of drought resistance which has a positive direct or indirect effect on plant productivity under drought stress (Ludlow and Muchow, 1990). Generally, when cells are subjected to slow dehydration, compatible solutes are accumulated in the cytosol, resulting in the maintenance of cell water content against the reduction in apoplastic water potential. The compatible solutes—various sugars, organic acids, amino acids, sugar alcohols, or ions (most commonly K^+)-differ with plant species and genera (Morgan, 1984). Most of the organic solutes used for osmotic adjustment originate in current assimilation and metabolism. Solutes used for cellular osmotic adjustment during stress may be partially used for regrowth upon recovery (McCree *et al.*, 1984). The accumulated solutes in the cytosol also allow the maintenance of symplast (Gupta and Berkowitz, 1987) and chloroplast (Robinson, 1987) volumes under water deficit. These specific effects together with cellular turgor maintenance are generally considered to support photosynthesis and growth under stress (Downton, 1983; Seemann *et al.*, 1986). The main solutes that are responsible for osmotic adjustment in rice under water deficit conditions were not elucidated. Rice does not accumulate glycine betaine as does barley and other plants (Ishitani *et al.*, 1993) because of a deficiency in choline monoxygenase and betaine aldehyde dehydrogenase (Rathinasabapathi *et al.*, 1993).

Rice accumulates proline (Dingkuhn *et al.*, 1991) and, at least in detached leaves, abscisic acid induced proline accumulation (Chou *et al.*, 1991). However, the extent of proline accumulation and its contribution to osmotic adjustment has not been evaluated. Since proline accumulation is proportional to the rate of plant water deficit, genotypic difference in proline accumulation may be simply a reflection of

respective differences in leaf water potential (Dingkuhn *et al.*, 1991; Nguyen *et al.*, 1997). The support of leaf turgor by osmotic adjustment in rice was well reflected in delayed leaf rolling when water deficit developed (Hsiao *et al.*, 1984). Leaf rolling, followed by leaf tissue death, seems to be a reliable index of turgor loss in rice induced by either a reduction in leaf water potential (O'Toole and Cruz, 1980) or turgor potential (Turner *et al.*, 1986). In the field, certain genotypes may show early leaf rolling because their leaf water potential is lowered, while others may show early rolling because of lack of osmotic adjustment (Dingkuhn *et al.*, 1989). Results indicated that leaf rolling and leaf death can be delayed by osmotic adjustment in rice (Hsiao *et al.*, 1984).

Henderson *et al.* (1993) have indicated some positive effects of osmotic adjustment in counteracting low leaf water potential, thereby, providing for greater retention of green leaf area. An increase in proline content by water stress has been suggested as a test of resistance to water stress (Palfi and Juhasz, 1971; Singh *et al.*, 1974). Mali and Mehta (1977) reported that drought-tolerant rice cv. TKM 1 and susceptible cv. Improved Sabarmati showed 5.4-fold and 1.2-fold increases in their free proline contents, respectively, at -10 bar osmotic potential. In contrast, Hanson *et al.* (1977) reported that drought-susceptible barley variety had higher accumulated proline than resistant variety.

Delauney and Verma (1993) argued that proline biosynthesis is controlled by Δ^1 -pyrroline-5-carboxylate synthetase (P5CS). This enzyme is regulated by proline via feedback inhibition. A loss of this feedback regulation was observed under water stress. It can be responsible for high accumulation rates of proline under stress conditions (Gzik, 1996). Proline accumulation in response to water deficit has been concerned with changes in concentration in the shoot or, more specifically, the leaves. However, proline accumulates in all organs of the intact plant during water deficit, although accumulation is most rapid and extensive in the leaves (Chen *et al.*, 1964; Barnett and Naylor, 1965; Singh *et al.*, 1973). Accumulation in the root occurs both later and less extensively than accumulation in the leaf, following the onset of a water deficit (Aspinall and Paleg, 1981).

Although total crop dry matter production is linearly and positively related to crop transpiration, this relationship is partly derived from the fact that the control of

both transpiration and CO₂ exchange is dependent on stomatal activity (de Wit, 1958). However, loss of water can also occur through nonstomatal pathways for which no return in CO₂ fixation is expected. An important nonstomatal pathway is the leaf cuticle (Nguyen *et al.*, 1997). The hydraulic permeability of the leaf surface may depend on the cuticle and epicuticular wax structure and composition (Svenningsson, 1988). When the water supply is adequate, the stomatal resistance of rice leaves is about 3 to 5 s cm⁻¹, and thus the stomata is the major path of water loss. Under water stressed conditions, however, rice stomates are closed partially or completely, and hence the cuticle becomes the major path of water loss. Under such conditions, minimizing water loss by having greater cuticular resistance should help the rice plant in keeping a better water economy. The cuticular resistance of rice leaves varies considerably among varieties, and high cuticular resistance appears to account in part for the drought resistance of rice varieties (Yoshida and Reyes, 1976). Because the hydraulic permeability of the leaf surface may depend on the cuticle and epicuticular wax structure and composition, Si was interested for the control over nonstomatal water loss from leaves.

Since Si influences water loss from plants largely by reducing cuticle transpiration (Jones and Handreck, 1967; Lewin and Reimann, 1969; Yoshida, 1975), Okuda and Takahashi (1965) grew rice plant (low-land rice variety Norin No. 22) in nutrient culture solutions ranging from 0 to 46.6 ppm Si (100 ppm SiO₂) and measured the transpiration rate at certain intervals for a 2-month period. With increasing Si in the solution, they found a consistent decrease in transpiration rate; in one instance, the rate decreased from 5.1 to 3.6 mL/g fresh wt/24 h for the nutrient solution containing 0 and 46.6 ppm Si, respectively. Horiguchi (1988) reported higher transpiration rate of -Si (control) plants than of +Si (treated) plants. These findings and other observations published elsewhere (Yoshida, 1975) demonstrate that by increasing Si content of rice plants, it may be possible to reduce their internal water stress (Savant *et al.*, 1987).

In plant breeding for drought resistance, a number of techniques of varying sophistications may be used to evaluate drought resistance. The most practical method is to simply plant in fields for a simulated upland or rainfed lowland crop in the dry season. However, field experiments on plant response to water stress usually

involve withholding irrigation. For many studies, however, water stress of soil-grown plant cannot be manipulated well enough. Soil heterogeneity further complicates the interpretation of field data as osmotic solutions are employed to impose water stress in plants by exposing the root system to the solutions (Hohl and Peter, 1991).

Polyethylene glycol (PEG) and mannitol are frequently used to control water stress but PEG appears to be better suited as an external osmoticum to analyze water relations in plants (Hohl and Peter 1991; Pandey and Agarwal, 1998). The addition of nonionic osmotic agents such as PEG to liquid nutrient media has been shown to closely mimic special levels of soil water stress. Furthermore, drought avoidance by growing deep-root cultivars is not possible because roots of all cultivars are uniformly exposed to the same osmotic stress. Several studies have focused on the use of PEG as an osmotic agent. PEG imposes water stress on plants by decreasing the water potential of the rooting medium, and thus the water potential of the plant. Because PEG is not readily taken up by the cells, it does not alter the ionic composition of the cell. The main concern about the use of PEG is the presence of toxic inorganic elements that presumably were residues of catalysts (such as aluminum) used in polymerization and organic contaminants such as formaldehyde. However, these impurities can be effectively removed by dialysis or through ion exchange columns (Cabuslay *et al.*, 1999). The regulation of proline accumulation in PEG (-1.5 MPa)-treated rice leaves was investigated. PEG treatment resulted in a decrease in relative water content, indicating that PEG treatment caused water stress in rice leaves. Proline accumulation caused by PEG was related to protein hydrolysis, an increase in ornithine- δ -amino- transferase activity, an increase in the content of ammonia, and an increase in the contents of the precursors of proline biosynthesis, glutamic acid, ornithine, and arginine (Hsu *et al.*, 2003).

In terms of the role of Si on upland rice under drought condition, information regarding drought resistance is still lacking. Therefore, this experiment is focused on the effects of Si application on drought resistance, and the relationship between Si content in plant tissues and drought resistance in upland rice in order to verify whether silicon content in rice plant tissues may be useful to selection criteria for breeding drought-resistant variety in upland rice.

3.2 Materials and methods

This experiment was conducted during September to October, 2006, at the nursery of Rajamangala University of Technology Lanna-Nan, Nan. Four upland rice varieties differing in Si uptake ability at tillering stage were used as plant materials in the present study. They were Hao (high Si content in leaf), IRAT191 (low Si content in leaf), SMGC90002-4 (high Si content in stem), and SMG9037-2-1-1-2 (low Si content in stem). These informations were received from Chapter 2. The treatments consisted of 0 and 200 ppm of Si application in culture solution. They were replicated thrice and the experiment was laid out in Completely Randomized Design. Seeds of four upland rice varieties were soaked in water for overnight at 25°C in the dark after sterilizing the surface with 0.1% sodium hypochlorite for 2 min. The seeds were then transferred to a net floated on Yoshida *et al.* (1976) solution in a plastic container. The composition of this nutrient solution contained the macronutrients 1.4 mM NH_4NO_3 , 0.3 mM $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 0.5 mM K_2SO_4 , 1.0 mM CaCl_2 , 0.8 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and the micronutrients 11 μM $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.1 μM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 19 μM H_3BO_3 , 0.2 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 μM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 36 μM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. On day 7, three seedlings of uniform size were transferred to a 3.5-L plastic pot containing one-half-strength Yoshida *et al.* (1976) solution with applied 0 or 200 ppm of Si. The pH of this solution was 5 and the nutrient solution was renewed every 7 days. After one month, the culture solution with various Si concentrations were added PEG 6000, giving -0.3 MPa of drought stress. The required amount of PEG 6000 was computed according to Lawlor (1970). Solution of PEG 6000 was purified by mixing with ion-exchange resin prior to adding nutrients. The experiment was terminated after 10 days when different drought among genotypes were visible.

Data collection

The performance of four rice varieties under different treatments was recorded through dry weight, relative water content (RWC), proline, stomatal resistance and Si content in leaf blade and stem tissues.

1) Dry weight: For each sample, rice plant samples were taken randomly from 5 plants of each variety. Then, these plant samples were separated into two parts.

First part was shoots and second part was roots. Each part of rice plant samples was then oven-dried for 72 hr at 80°C and the dry weight of each organ was measured after drying sample.

2) RWC: For each sample, 5 pieces of the second-youngest fully-expanded leaf were weighed and cut into 5-cm segments and then allowed to rehydrate inside a refrigerator overnight. The following day, samples were blotted dry and turgid weight immediately determined. The leaves were then oven-dried for 2 days at 80°C. Leaf RWC was then calculated according to Basnayake *et al.* (1993).

$$\text{RWC} = \frac{(\text{FW}-\text{DW})}{(\text{TW}-\text{DW})} \times 100$$

when FW = Fresh weight of leaves

DW = Dry weight of leaves

and TW = Turgid weight of leaves

3) Proline accumulation: For each sample, 5 segments of the youngest fully-expanded leaf from 5 plants of each genotype were homogenized in 10 mL of 3% sulfosalicylic acid and the homogenate was centrifuged. The content of proline was determined according to the method of Bates *et al.* (1973). 1 mL filtrate was mixed with equal volumes of acetic acid and ninhydrin (1.25 mg ninhydrin, 30 mL of glacial acetic acid, 20 mL 6 M H₃PO₄) and incubated for 1 hr at 100°C, the reaction was stopped by placing the test tubes in ice-cold water. The samples were rigorously mixed with 3 mL toluene. After 50 min, the light absorption of the toluene phase was estimated at 520 nm. The proline content was determined using a standard curve and expressed as μmol g⁻¹ of initial fresh weight.

4) Stomatal resistance: For each sample, stomatal resistance was measured at the youngest fully-expanded leaf from 5 plants of each genotype between 11.00 to 12.00 am by polometer (model AP4 produced by Delta-T devices-Cambridge-UK).

5) Si content in leaf blade and stem tissues: For each sample, rice plant samples were taken randomly from 5 plants of each genotype. Then, these plant

samples were separated into two parts. First part was stems (stems and leaf sheaths), and second part was leaf blades only. Silicon content in leaf blade and stem tissues were determined by the AID method (Elliott and Snyder, 1991) as described before.

Statistical analysis

Data were analysed statistically by ANOVA, with subsequent comparison of means by LSD. All of statistical analyses were done by using commercial software (Statistix V. 7.1, Analytical Software, Inc.).

3.3 Results

3.3.1 Dry weight

Table 3.1 shows that dry weight of upland rice plant parts which included root, shoot and total dry weights were significantly increased by Si application in culture solution under drought condition ($P < 0.01$). However, no significant differences were observed in root, shoot and total dry weights among the varieties ($P < 0.01$). Furthermore, significant interaction was not observed between the Si application and variety on the root and shoot dry weights, except the total dry weights ($P < 0.01$). The interaction between the Si application and variety on the total dry weight of rice plants could be classified into four groups. First group, SMGC 90002-4 and Hao varieties which were grown in the Si-applied culture solution showed highest total dry weight of rice plants. Second group, the intermediate total dry weight of rice plants was SMG 9037-2-1-1-2 and IRAT 191 varieties which were grown in the culture solution with 0 and 200 ppm Si application. Third group, the low total dry weight of rice plant was Hao variety which was grown in the culture solution without Si application. Fourth group, the least total dry weight of plant rice was SMGC 90002-4 variety which was grown in the culture solution without Si application.

Table 3.1 Effect of Si application on root dry weight, shoot dry weight, and total dry weight of var. Hao, IRAT 191, SMG 9037-2-1-1-2 and SMGC 90002-4 grown under drought condition.

Variety	Si concentration in culture solution (ppm)	Root dry weight (g plant ⁻¹)	Shoot dry weight (g plant ⁻¹)	Total dry weight (g plant ⁻¹)
Hao	0	0.0904	0.2114	0.3018 ^{bc}
	200	0.0970	0.2708	0.3678 ^a
IRAT 191	0	0.0893	0.2315	0.3209 ^{ab}
	200	0.0930	0.2686	0.3616 ^{ab}
SMG 9037-2-1-1-2	0	0.0910	0.2341	0.3251 ^{ab}
	200	0.1016	0.2561	0.3576 ^{ab}
SMGC 90002-4	0	0.0748	0.1712	0.2461 ^c
	200	0.1063	0.2656	0.3719 ^a
<u>Means for Si conc.</u> [†]	0	0.0864 ^x	0.2121 ^x	0.2985 ^x
	200	0.0995 ^y	0.2652 ^y	0.3647 ^y
<u>Means for variety</u> [†]	Hao	0.0937	0.2411	0.3348
	IRAT 191	0.0912	0.2500	0.3412
	SMG 9037-2-1-1-2	0.0963	0.2451	0.3414
	SMGC 90002-4	0.0906	0.2184	0.3090
<i>F</i> -test (Si)		**	**	**
<i>F</i> -test (var.)		ns	ns	ns
<i>F</i> -test (Si x var.)		ns	ns	*

[†] Data are the means of three replications. Different letters in the table indicate significant differences by LSD ($P < 0.05$ or 0.01 followed to *F*-test).

**, * and ns = significant at the 0.01, 0.05 probability levels and not significant by ANOVA, respectively.

3.3.2 Stomatal resistance of leaves

As shown in Table 3.2, the stomatal resistance of leaves was significantly decreased by Si application in culture solution under drought condition ($P < 0.01$). Applying 200 ppm of Si in culture solution decreased the stomatal resistance of leaves from 8.7667 to 4.2542 s cm⁻¹, compared with no Si application or decreased about 51.47%. While variation among varieties was significant for the stomatal resistance of leaves ($P < 0.01$), IRAT 191 variety showed the highest stomatal resistance of leaves and followed by SMG 9037-2-1-1-2, SMGC 90002-4 and Hao, respectively. However, SMG 9037-2-1-1-2, SMGC 90002-4 and Hao were not significantly different.

There was a significant interaction between the Si application and variety on the stomatal resistance of leaves ($P < 0.01$). When the Si was applied in the culture solution, the stomatal resistance of leaves was decreased in all varieties and they showed a 31.90 to 65.66% relative decrease in the stomatal resistance of leaves. The strongest effect of Si application on the stomatal resistance of leaves was found in SMG 9037-2-1-1-2 variety (65.66%) and slight effect in SMGC 90002-4 (31.90%). However, the stomatal resistance of leaves was highest when IRAT 191 variety was grown in the culture solution without Si application.

3.3.3 Relative water content

Under drought condition, RWC in leaves was significantly increased by Si application in culture solution ($P < 0.01$) (Table 3.2). It increased about 6.72% when compared with no Si application. For effect of variety, variations among varieties were significant for RWC in leaves ($P < 0.01$) and they were classified into three groups. First group, Hao variety showed highest RWC in leaves. Second group, varieties with the intermediate RWC in leaves were SMGC 90002-4 and SMG 9037-2-1-1-2. And third group, IRAT 191 showed least RWC in leaves. However, significant interaction was not observed between the Si application and variety.

Table 3.2 Effect of Si application on stomatal resistance, relative water content, and proline accumulation of var. Hao, IRAT 191, SMG 9037-2-1-1-2 and SMGC 90002-4 grown under drought condition.

Variety	Si concentration in culture solution (ppm)	Stomatal resistance (s cm ⁻¹)	Relative water content (%)	Proline accumulation (μ mole g ⁻¹ of fresh weight)
Hao	0	7.3333 ^c	82.637	0.7117 ^{bc}
	200	3.6333 ^e	93.313	0.4547 ^e
IRAT 191	0	11.367 ^a	80.027	1.8073 ^a
	200	5.4000 ^d	81.620	0.7333 ^{bc}
SMG 9037-2-1-1-2	0	9.3667 ^b	81.740	1.0497 ^a
	200	3.2167 ^e	85.703	0.6433 ^{cd}
SMGC 90002-4	0	7.0000 ^c	79.253	0.8033 ^b
	200	4.7667 ^{de}	89.907	0.5387 ^{de}
<u>Means for Si conc.</u> [†]				
	0	8.7667 ^x	80.914 ^x	1.0930 ^x
	200	4.2542 ^y	87.636 ^y	0.5925 ^y
<u>Means for variety</u> [†]				
	Hao	5.4833 ^p	87.975 ^p	0.5832 ^p
	IRAT 191	8.3833 ^q	80.823 ^q	1.2703 ^f
	SMG 9037-2-1-1-2	6.2917 ^p	83.722 ^{pq}	0.8465 ^q
	SMGC 90002-4	5.8833 ^p	84.580 ^{pq}	0.6710 ^p
<i>F</i> -test (Si)		**	**	**
<i>F</i> -test (var.)		**	*	**
<i>F</i> -test (Si x var.)		**	ns	**

[†] Data are the means of three replications. Different letters in the table indicate significant differences by LSD ($P < 0.05$ or 0.01 followed to *F*-test).

**, * and ns = significant at the 0.01, 0.05 probability levels and not significant by ANOVA, respectively.

3.3.4 Proline accumulation in leaves

For this study, proline accumulation in leaves was compared between plants grown in culture solution with 0 and 200 ppm Si application under drought condition. They were significantly decreased by Si application in culture solution and decreased from 1.0930 to 0.5925 μ mole g^{-1} of fresh weight (Table 3.2) or decreased about 45.79%. In addition, the difference in proline accumulation in leaves among varieties was highly significant ($P < 0.01$) and they were classified into three groups. First group, Hao and SMGC 90002-4 varieties showed least proline accumulation in leaves. Second group, variety with the intermediate proline accumulation in leaves was SMG 9037-2-1-1-2. And third group, IRAT 191 showed highest proline accumulation in leaves.

There was a significant interaction between the Si application and variety on the proline accumulation in leaves ($P < 0.01$). The results showed that accumulation was decreased in all varieties by Si application in culture solution ($P < 0.01$) and relatively decreased from 59.42 to 32.94%. The strongest effect of Si application on the proline accumulation in leaves was found in IRAT 191 variety (59.42%) but slight effect was observed in SMGC 90002-4 (32.94%). However, highest proline accumulation in leaves was found in IRAT 191 variety which was grown in the culture solution without Si application.

3.3.5 Si content in leaf blade and stem tissues

The results show that the Si content in leaf blade and stem tissues increased significantly by Si application in culture solution under drought condition (Table 3.3). The Si content in leaf blade tissues was relatively high compared with stem tissues. There were highly significant differences in the Si contents among the varieties for both Si content in leaf blade and stem tissues. The highest Si content in leaf blade tissues varieties were Hao, followed by SMGC 90002-4, SMG 9037-2-1-1-2 and IRAT 191. SMGC 90002-4 variety showed the highest Si content in stem tissues but significant differences were not observed among Hao, IRAT 191 and SMG 9037-2-1-1-2 varieties.

Table 3.3 Effect of Si application on Si content in leaf blade, and stem tissues of var. Hao, IRAT 191, SMG 9037-2-1-1-2 and SMGC 90002-4 grown under drought condition.

Variety	Si concentration in culture solution (ppm)	Si content in leaf blade tissues (mg g ⁻¹)	Si content in stem tissues (mg g ⁻¹)
Hao	0	0.0038 ^e	0.0024 ^c
	200	38.433 ^a	7.4683 ^b
IRAT 191	0	0.0024 ^e	0.0015 ^c
	200	18,244 ^d	6.2900 ^b
SMG 9037-2-1-1-2	0	0.0031 ^e	0.0020 ^c
	200	24.367 ^c	5.8550 ^b
SMGC 90002-4	0	0.0024 ^e	0.0016 ^c
	200	27.083 ^b	14.213 ^a
<u>Means for Si conc.</u> [†]			
	0	0.0029 ^x	0.0019 ^x
	200	27.032 ^y	8.4567 ^y
<u>Means for variety</u> [†]			
	Hao	19.219 ^p	3.7354 ^q
	IRAT 191	9.1234 ^s	3.1458 ^q
	SMG 9037-2-1-1-2	12.185 ^r	2.9285 ^q
	SMGC 90002-4	13.543 ^q	7.1074 ^p
<i>F</i> -test (Si)		**	**
<i>F</i> -test (var.)		**	**
<i>F</i> -test (Si x var.)		**	**

[†] Data are the means of three replications. Different letters in the table indicate significant differences by LSD ($P < 0.01$).

** = significant at the 0.01 probability level by ANOVA.

There was significant interaction between the Si application and variety on both Si content in leaf blade and stem tissues ($P < 0.01$) (Table 3.3). Si contents increased in all varieties when applied Si in the culture solution. The strongest effect of Si application on the Si content in leaf blade tissues was found in Hao variety, followed by SMGC 90002-4, SMG 9037-2-1-1-2 and IRAT 191. Si content in stem tissues was different from leaf blade tissues, the strongest effect of Si application was found in SMGC 90002-4 variety but significant differences were not observed among Hao, IRAT 191 and SMG 9037-2-1-1-2 variety.

3.4 Discussion

The results show that under drought condition, supplemental Si application in the rice culture solution will ameliorate the decrease of stomatal resistance, proline accumulation in leaf, and lead to the increase of dry weight, relative water content, and Si content in shoot tissues. These results demonstrated that under drought stress conditions, upland rice plants growing in Si-applied culture solution could reduce the transpirational loss of water to maintain higher RWC or water potential. As the stomatal resistance and relative water content were closely associated with the status of the plants, these identifications could be quantified and used as criteria for water stress evaluation. Under drought stress, the plant loses more water than it absorbs through its root, it suffers internal moisture stress, the stomata partially or entirely close, conserving water and, hence, preventing loss of internal plant moisture (Yoshida, 1975). The stomatal resistance is the resistance of the leaf to moisture loss through the stomata, can be used as a measure of internal moisture stress, and has negative relationship with the transpiration rate (Yoshida, 1975). Relative water content is the water content in proportion to that at full turgor and a commonly-used measure of water status in plant (Turner, 1986). These reasons were affirmed by the proline accumulation in leaves because under drought stress condition, accumulation was decreased by Si application in the culture solution. The content of proline was usually found to increase under water stress (Yoshida *et al.*, 1974). Thus, these results indicated that Si application enhanced the resistance to drought in upland rice. As stomata act as regulators for CO₂ exchange, as well as regulators of water loss,

water deficit is sufficient to close stomata and must also depress photosynthesis activity (Begg and Turner, 1976). Based on these facts, the Si application in culture solution could maintain the photosynthesis activity for the increase in dry weight under drought condition.

Table 3.4 Correlations among stomatal resistance (SR), relative water content (RWC), root dry weight (RDW), shoot dry weight (SDW), total dry weight (TDW), proline accumulation (P), Si content in stem tissues (Si-C) and Si content in leaf blade tissues (Si-L) grown under drought condition.

Traits	SR	RWC	RDW	SDW	TDW	P	Si-C
RWC	-0.58**						
RDW	-0.41*	0.43*					
SDW	-0.40	0.56**	0.58**				
TDW	-0.44*	0.58**	0.74**	0.98**			
P	0.86**	-0.51**	-0.25	-0.24*	-0.27*		
Si-C	-0.70**	0.61**	0.59**	0.60**	0.65**	-0.57**	
Si-L	-0.82**	0.74**	0.52**	0.67**	0.69**	-0.64**	0.83**

** and * = significant at the 0.01 and 0.05 probability levels, respectively.

However, statistical analysis indicated that under drought condition, Si content in leaf blade and stem showed negative correlation with stomatal resistance and positive correlation with relative water content (Table 3.4). These results suggested that the Si content in rice shoots will enhance the drought resistance in upland rice plant. As transpiration from the leaves occurs mainly through the stomata and partly through the cuticle and Si was deposited beneath the cuticle of the leaves forming a Si-cuticle double layer (Ma and Takahashi, 2002; Ma, 2004), there was a possibility that the Si content in rice shoots led to decrease in water loss from cuticle to maintain the high leaf water potential and would mask the increasing transpiration from the

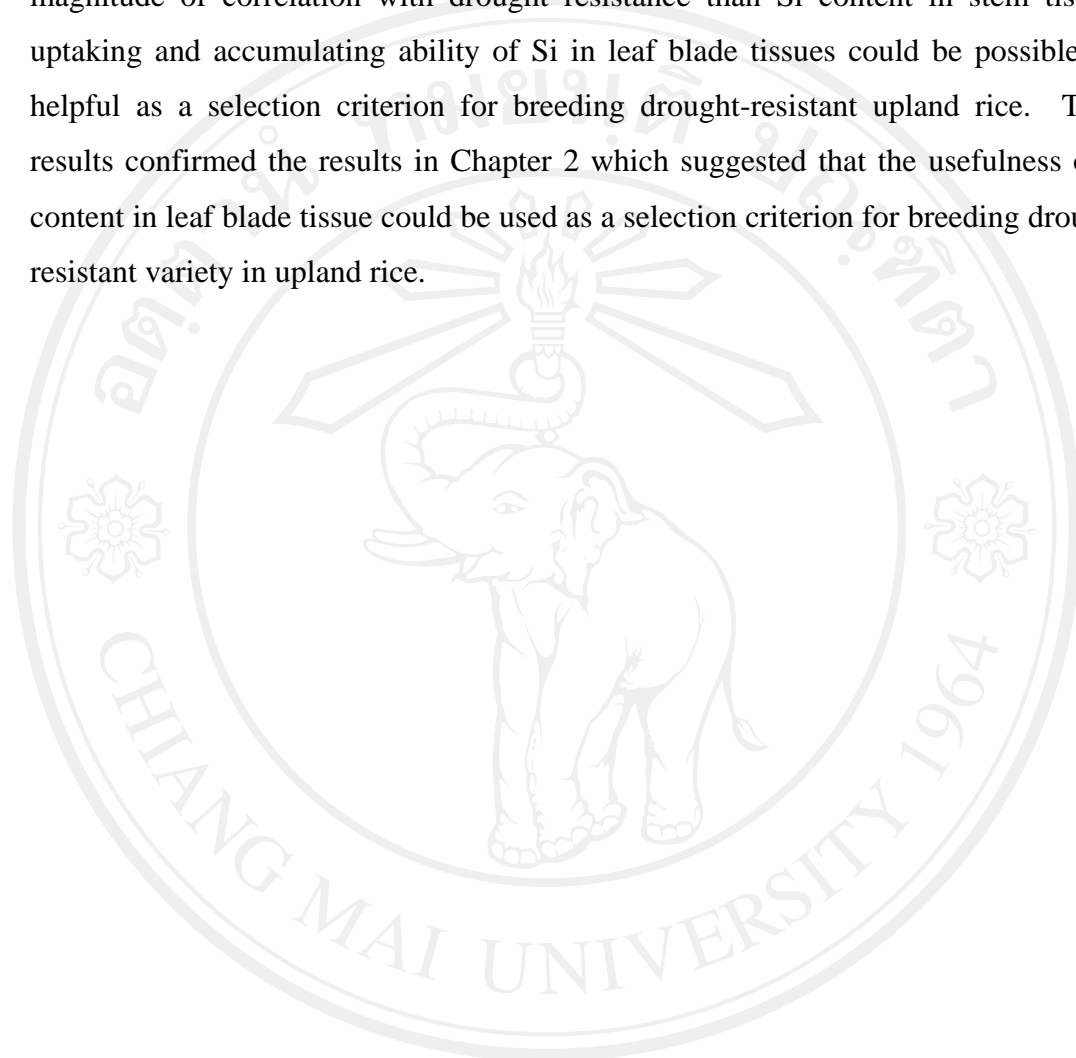
stomata. These results were similar to the reports of Ma *et al.* (2001), Ma and Takahashi (2002), Hatori *et al.* (2005). These results clearly indicated that the water loss through transpiration at leaf stomata and the cuticle might be minimized by Si deposition in rice plant organs.

In addition, the results show that significant variations were observed among the varieties for stomatal resistance, relative water content, proline accumulation and Si content in leaf blade and stem tissues (Tables 3.2 and 3.3), indicating that there were some varieties which had higher Si uptake ability than others or the ability of Si uptake depended on genetic variation of upland rice. These results agreed with Garrity *et al.* (1984), Majumder *et al.* (1985), Winslow (1992) and Deren *et al.* (1992). As described earlier that the Si content in rice shoots will enhance the resistance to drought in upland rice plant, these results suggested that varieties possess different degrees of drought resistance (Ma and Takahashi, 2002). Hao could maintain higher water content than the other varieties under drought condition. However, there were no significant differences in dry weight among the varieties. Hence, it clearly indicated that varieties which were different in ability of growth and adaptation under drought condition (Fukai and Cooper, 1996), not only depended on Si uptake and accumulation in shoot but also Si could maintain the photosynthesis activity for increasing in dry weight (Matoh *et al.*, 1991; Ma and Takahashi, 2002; Hattori *et al.*, 2005).

Moreover, there were significant interactions among the Si application and variety on stomatal resistance, proline accumulation, Si content in leaf blade and stem tissues. Since Si x variety interactions are expressed as a change in the relative performance of varieties with applied Si in the culture solution, therefore, supplemental Si application which enhances the uptaking ability and accumulating Si in upland rice genotypes (Ma and Takahashi, 2002) can be used to improve the drought resistance.

Results of this study could be concluded that under drought stress condition, supplemental Si application in the rice culture solution may be useful as a criterion to improve the drought resistance of upland rice via the enhancement of water content in plant. However, the abilities of Si uptake in upland rice depend both on genetic variation and Si concentration in culture media. Since Si content in leaf blade and

stem tissues of upland rice varieties showed predominant characters in connection with drought resistance, especially Si content in leaf blade tissues showed a higher magnitude of correlation with drought resistance than Si content in stem tissues, uptaking and accumulating ability of Si in leaf blade tissues could be possible and helpful as a selection criterion for breeding drought-resistant upland rice. These results confirmed the results in Chapter 2 which suggested that the usefulness of Si content in leaf blade tissue could be used as a selection criterion for breeding drought-resistant variety in upland rice.



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

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