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INDUCTION OF SALT TOLERANCE IN RICE (*Oryza sativa* L.) BY BRASSINOSTEROIDS

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Dedicated

to the memory of my late father and brother

Induction of Salt Tolerance in Rice (*Oryza sativa* L.) by Brassinosteroids

Abstract

Salinity is one of the most serious constraints of rice production in the Mekong Delta of Vietnam. Currently, recommended strategies to overcome the adverse effects of salt stress include the use of tolerant cultivars, ameliorative water management and diverse cultural practices. However, none of these strategies is fully effective under the diverse environmental conditions or adoptable by farmers in the Mekong Delta. For improvement of quality parameters in rice grown under salt stress conditions, biological processes have been established on the basis of plant protectants, the brassinosteroids. They have been demonstrated to show effects in yield increase and its parameters in the induction of salt tolerance and in adaptation mechanisms against salt-stress. Recent findings on the effects of brassinosteroids on the stress tolerance of crops open new avenues to address the salinity problems in rice (*Oryza sativa* L.).

The effects of an application of brassinosteroid "24-epibrassinolide" (EBl) on growth, yield and physiological traits of salinity-tolerant (MTL119) and salinity-sensitive (IR28) rice cultivars were studied under controlled growing conditions in hydroponics and potted soil. The effect of brassinosteroid treatment on the salinity tolerance of rice during the germination involved seed soaking in 0, 0.5, 1.0 and 1.5 mg l^{-1} of EBl solution with 0 and 100 mmol NaCl addition. The effect of brassinosteroid treatment on the salinity tolerance of rice during the seedling stage involved seed soaking in 0 and 1 mg l^{-1} of EBl solution and subsequent germination in nutrient solution with 0 and 100 mmol NaCl addition. The effect of brassinosteroid treatment on the salinity tolerance of rice during vegetative and reproductive growth stages involved seed soaking and the foliar application of EBl solution at a concentration of 1 mg l^{-1} of EBl at the early vegetative (25 days after sowing) and the panicle initiation stages of rice grown in potted soil. Salinity levels of 0, 50 and 100 mmol NaCl (approx., 0, 5 and 10 dSm^{-1}) were applied between 30 and 51 days after seeding.

Seed soaking with EBl improved both the earliness and the total germination (up to 50%) of cultivar MTL119 but had no effect on the salinity-sensitive cultivar IR28. While EBl application affected neither shoot or root growth nor the chlorophyll content of 14 day-old seedlings, it tended to improve the salinity tolerance of the seedlings. This trend was associated with a significant increase in the leaf proline content from 1.23 in control to 2.02 µmol (64%) in EBl-treated plants. EBl application during the vegetative and reproductive growth stages had little or no significant effect on the salinity tolerance of both varieties as measured by yield and yield parameters or a range of physiological parameters (leaf area, chlorophyll content, photosynthesis). However, most parameters, including the grain yield, tended to increase by up to 30% in the salt tolerant cultivar MTL119 and by up to 14% in the salt-sensitive cultivar IR28. However, EBl application significantly enhanced the number of filled grains by 20% in cultivar MTL119 under mild salinity stress of 5 dS m^{-1}. This tendency was accompanied by a significant increase in leaf proline concentration (48-70%).

It may be concluded that brassinosteroids appear to improve the salinity tolerance of some rice cultivars under mild salt stress. The physiological mechanism for this enhanced stress tolerance has been shown to involve the accumulation of proline in the cell of leaves. Further studies are needed to confirm the positive effect of EBl application on salt stress tolerance and the positive mechanism of proline accumulation and must involved field studies using a wider range of rice germplasm and more diverse environmental conditions.

Key Words: 24-epibrassinolide, *Oryza sative* L., Proline, Stress tolerance

Die Induktion von Salztoleranz beim Reis (*Oryza sativa* L.) unter Einfluss von Brassinosteroiden

Salinität ist im vietnamesischen Mekong Delta einer der begrenzenden Faktoren des Reisanbaus. Die derzeitig angewendeten Strategien zur Minimierung der durch Salzstress hervorgerufenen negativen Effekte beinhalten den Einsatz von toleranten Sorten, verbessertem Wassermanagement, sowie einer Anzahl verschiedener Anbaupraxen. Keine dieser Strategien jedoch erzielt unter den gegebenen Umweltbedingungen zufriedenstellende Erfolge. Zudem ist der Einsatz bzw. die Umsetzung dieser Konzepte für lokale Landwirte des Mekong Deltas nur bedingt realisierbar. Für die Verbesserung von Qualitätsparametern von unter Salinität angebautem Reis wurden auf der Basis von einem Pflanzenstärkungsmittel, den Brassinosteroiden, biologische Verfahren etabliert. Es wurde nachgewiesen, dass im Zusammenhang mit Salzstress und der damit verbundenen Induktion von Salztoleranz bzw. entsprechenden Adaptionsmechanismen durch Brassinosteroide, Ertragsparameter beeinflusst werden und somit der Ertrag gesteigert werden kann. Neuere Erkenntnisse, die eine Wirkung von Brassinosteroiden auf die Salztoleranz von landwirtschaftlich relevanten Kulturpflanzen aufweisen, eröffnen eine neues und breites Forschungsfeld und steuern gleichermaßen eine Verminderung der Salzproblematik beim Anbau von Reis (*Oryza sativa* L.) an.

Die Effekte einer Applikation des Brassionsteroid „24-epibrassinolide“ (EBI) auf das Wachstum, den Ertrag und die physiologischen Eigenschaften der salztoleranten Varietät MTL119 und der salzsensitiven Varietät IR28 wurden unter kontrollierten Klimabedingungen in Flüssigmedium, wie auch in Versuchen mit Topfboden experimentell untersucht. Die Experimente zum Einfluss von Brassinosteroiden auf die Salztoleranz während des Keimlingsstadiums beinhalteten das Quellen der Samen in 0; 0,5; 1,0 und 1,5 mg l^{-1} EBI Lösung und die Zugabe von 0 bis 100mmol NaCl. Bei den Untersuchungen zum Einfluss von Brassinosteroiden auf die Salztoleranz während der vegetativen und der reproduktiven Entwicklungsphase wurden die Samen in 1,0 mg l^{-1} EBI vorgequollen und eine Blattapplikation von 1,0 mg l^{-1} EBI in einem frühen vegetativen Stadium (25 Tage nach Aussaat) verabreicht. Desweiteren wurde eine Blattapplikation während der späteren Ährenformationsphase von in Topfboden aufgezogenen Reispflanzen vorgenommen. Salzkonzentrationen zwischen 0,5 und 100 mmol NaCl (ca. 0,5 und 10 dSm^{-1}) wurden ab dem 30. bis 51. Tag nach der Aussaat appliziert.

Das Vorquellen der Samen in EBI verbesserte sowohl die Geschwindigkeit der Keimung, sowie auch die effektive Keimrate (um bis zu 50%) der Varietät MTL119, zeigte jedoch keinen Effekt auf die salzsensitive Varietät IR28. Obwohl die EBI-Applikationen weder einen Effekt auf den Spross, noch auf die Wurzeln bzw. auf den Chlorophyllgehalt 14 Tage alter Keimlinge aufwiesen, ließ sich dennoch eine Tendenz einer Verbesserung der Salztoleranz erkennen. Dieser Trend wurde mit einem signifikanten Anstieg des Prolingehalts mit 1,23 µmol in Kontrollpflanzen auf bis zu 2,02 µmol (64%) in Blättern von mit EBI behandelten Pflanzen in Verbindung gebracht. Die Messung von Ertrag und Ertragsparametern bzw. physiologischen Parametern (Blattfläche, Chlorophyllgehalt, Photosynthese) wurden als Indizien für die Salztoleranz verwendet und ergaben nach EBI-Behandlung keine bzw. schwache Wirkungen auf die Salztoleranz während der vegetativen bzw. generativen Phase beider Varietäten. Es konnte jedoch nachgewiesen werden, dass für eine Vielzahl von Parametern, wie z.B. für den Kornertrag, ein Anstieg von bis zu 30 % bei der salztoleranten Varietät MTL119 und um bis zu 14% bei der salzsensitiven Varietät IR 28 beobachtet werden konnte. Zudem wurde nachgewiesen, dass die Anzahl der gefüllten Körner der Varietät MTL119 unter milden Salzstressbedingungen (5 dSm^{-1}) nach einer Behandlung mit EBI um 20% gestiegen ist. Diese Tendenz wurde begleitet von einer signifikanten Zunahme des Prolingehalts in den Blättern (48-70%).

Die Ergebnisse einer Behandlung mit Brassinosteroiden weisen darauf hin, dass die Toleranz gegenüber Salz in manchen Reisvarietäten unter mildem Salzstress erhöht werden kann. Zudem ist erkennbar, dass der physiologische Mechanismus, der sich hinter dieser Salzstresstoleranz verbirgt, mit einer Akkumulation von Prolin in den Zellen der Reisblätter einhergeht. Weitere Studien werden benötigt, um den positiven Effekt von EBI auf die Salzstresstoleranz und den damit einhergehenden Anstieg des Prolingehalts zu untermauern. Hierbei müssen auch insbesondere Feldversuche, basierend auf einer Vielzahl von unterschiedlichen Reisgermplasmen und variierenden Umweltbedingungen, verknüpfend untersucht werden.

Table Content

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List of abbreviations

ABA	abscisic acid
BRs	brassinosteroids
DBS	days before sowing
DAS	days after sowing
DBS-S	days before salt-stress
DAS-S	days after salt-stress
EBl	24-epibarssinolide
EC	electric conductivity
ET	electron transport
GA	gibberellic acid
Gs	stomatal conductance
LAI	leaf area index
Mg ha^{-1}	megagram per ha (ton ha^{-1})
Pn	CO_2 assimilation
QE	quantum efficiency

1. INTRODUCTION

1.1. Salinity constraints to rice production in Vietnam

Based on the FAO/UNESCO Soil Map of the World, saline soils cover some 397 million ha and sodic soils some 434 million ha of the global land area. Of the nearly 1,500 million ha of dry agricultural land, 32 million (2.1%) are salt-affected, while of the currently 230 million ha of irrigated agricultural land, 45 million ha (19.5%) are salt-affected, to varying degrees by anthropogenic-induced processes (Oldeman, 1991). Nearly one third of this irrigated land area is used for the production of lowland rice.

Due to the formation of an aerenchyma rice can adapt better to flooded conditions than other annual crops, which cannot survive under water logged conditions for more than 5 days (White, 1996). However, salinity is one of the most serious biophysical constraints of rice production in many rice-producing areas of the world. An estimated area of 150 million ha of current and potential rice land in the tropics and subtropics is affected by salinity (Massoud, 1974). In South and South East Asia alone, nearly 49 million ha are affected by soil salinity and another 12 million ha are affected by soil alkalinity or sodicity (Akbar and Ponnamperuma, 1982).

Vietnam is situated in tropical monsoon climate region, with two separate seasons: the rainy (May-October) and dry (November-April) season. Fresh water lacks critically in the rainfed areas during the dry season because 90% of annual rainfall falls during the rainy season and 10% falls in the dry season. On the other hand, Vietnam has a long coast (3,400 km) and hence large areas (2 million ha) are affected by salinity (Vo, 1995; FAO, 2000). Especially the Mekong Delta in the South-West of the country is a lowland region with an altitude of less than 2 m above mean sea level. Therefore, the effect of saline intrusion is more serious in the Mekong Delta than in other regions of Vietnam. The seawater intrusion reaches only 15 km in-land in the Red River Delta, but can reach 40-50 km (0.3-0.4% salt concentration ~ EC= 5-7 dS m^{-1}) and 100 km (0.1% ~ 1.7 dS m^{-1}) in the Mekong

River Delta (FAO, 2000; MARD, 2005). Of a total of 3.9 million ha of land, approximately 1 million ha are affected by tidal flooding and 1.7 million ha by salt water intrusion (Massoud, 1974; Wassmann *et al.*, 2004).

Rice is the dominant crop in the Mekong Delta with 2 million ha (double crops per year ~ 4 million ha year^{-1}), or 53% of the countrys rice-growing area. However, 0.7 million ha of lowland rice are affected by salinity, particularly during the dry season (Bui and Nguyen, 2004) and annually, salt-water intrusion severely damages the agricultural production. According to an investigation of the Vietnamese Ministry of Agriculture and Rural Development (MARD), the economic loss by salt-water intrusion in 2005 amounted to 45 million USD, equivalent of 1.5 % of annual rice production in the Mekong Delta (MARD, 2005). With the use of short-cycled rice genotypes and the implementation of other flood-escape strategies, salinity damage is now considered more important than flood damage to rice production in the Mekong Delta (Bui and Nguyen, 2004).

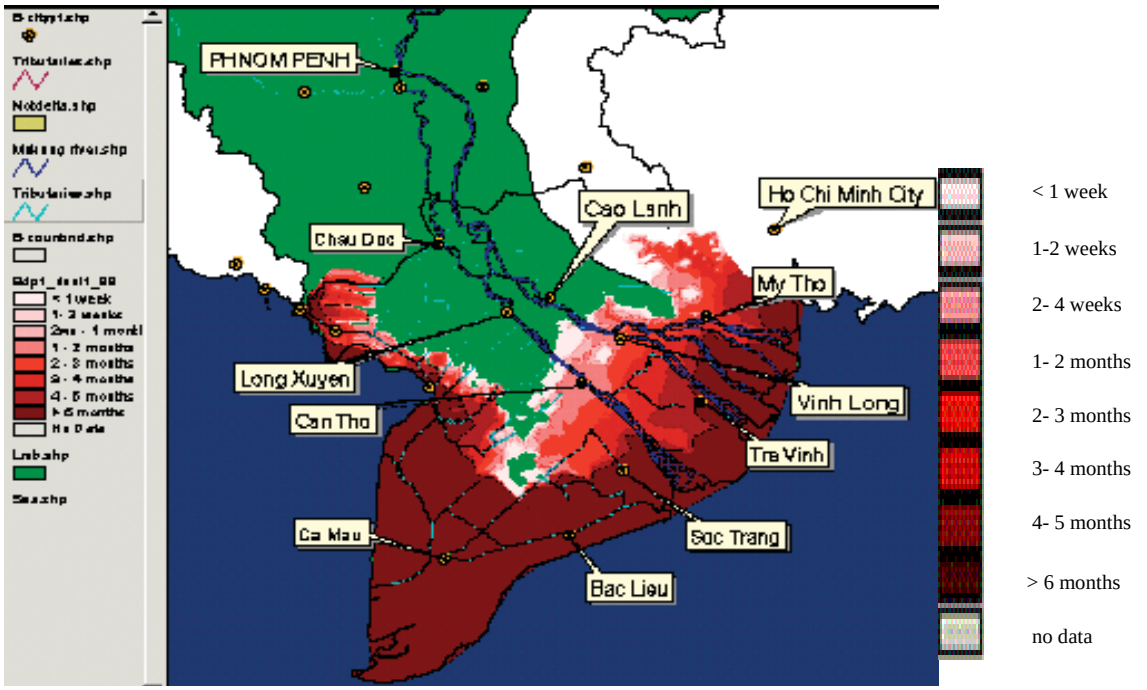


Figure 1. Mekong Delta: Simulation of salt-water intrusion during the dry season of 1998 (adapted from MRC, 2005). The map shows the duration of salinity levels greater than 1 g l^{-1}. The area affected 28,500 km^2 per 39,000 km^2 (Vietnamese part of the Mekong Delta).

Furthermore, in the future the seawater intrusion is expected to become more serious (Figure 2) as the result of increasing diversions of water for dry season irrigation, both in Vietnam and in countries upstream (White *et al.*, 1996). The decrease of the flow volume of the Mekong River during the dry season is further exacerbated by the construction of hydroelectric power plants by other upstream countries (MRC, 2002), as well as possibly by the effects of global warming, which is forecast to induces an increase of the sea level in Southeast Asia by 2 mmyear^{-1} (Tuong, 2001).

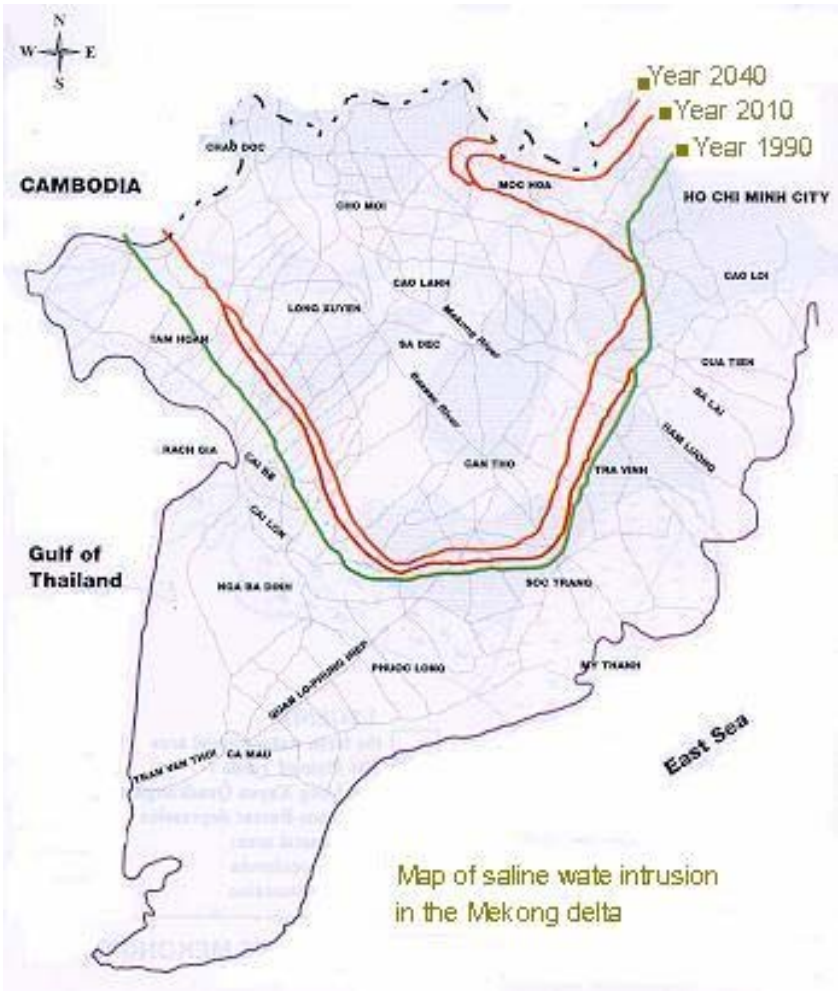


Figure 2. Trends of saline water intrusion to the Mekong Delta (adapted from Nguyen *et al.*, 1998)

Consequently, fresh water critically lacks during the dry season for the non-irrigated areas in the Mekong Delta, which occupy an estimated 30% of the rice production area (Bui and Nguyen, 2004). In these areas, rice can only be cultivated during the rainy season and strongly depends on the rainfall. Generally, there is not enough fresh water in the early or late rainy season when saline water (about 0.3 % of NaCl, approx. 5 dS m^{-1}) can intrude into the fields and directly affect the yield of rice or result in accumulating salt in the soil, which may damage the following crop. It may be concluded that the salinity problematic in rice production of the Mekong Delta is serious and likely to increase in the foreseeable future.

1.2. Salinity effects on plants

Salinity is the result of either the intrusion of seawater into the agricultural area, or of a raise in salt-affected groundwater. According to the FAO (Food and Agriculture Organization), salinity may be classified into five levels, which are presented in Table 1.

Table 1. Indicative soil salinity classes and implications for crop performance (adapted from FAO, 2001)

EC at 25 °C	**Salt Concentration**		**Effect on crops**
(dS m^{-1})	(cmol l^{-1})	(%)	
<2.0	<2		mostly negligible
2.0 - 4.0	2 - 4	<0.15	some damage to sensitive crops
4.0 - 8.0	4 - 8	0.15 - 0.35	serious damage to most crops
8.0 - 15.0	8 - 15	0.35 - 0.65	only tolerant crops succeed
>15	>15	>0.65	few crops survive

EC: Electric conductivity

Some researchers found that low salinity at the early developmental stages can have a stimulatory effect on plant growth. However, high salinity levels at any growth stage will inhibit the growth and yield of rice (Flowers and Yeo, 1981; Khatun and Flowers, 1995a, 1995b). High salinity is detrimental to plant growth as it causes

1) nutritional disorders by decreasing the uptake of cations, such as potassium and calcium, but also of anions such as phosphorus and nitrate (Asch *et al*., 2000)
2) ion cytotoxicity mainly due to elevated concentrations of Na^+, Cl^-, plus SO^-_4. and
3) osmotic stress (Zhu, 2001).

The combined effects of ion toxicity, osmotic stress, and nutritional disorders may lead to a metabolic imbalance, resulting in oxidative stress.

Most plants suffer from salt injury at electric conductivity (EC) values exceeding 4 dS m^{-1}, while some tolerant crops can withstand much higher concentrations. Crop yield markedly decreases with an increase in salt concentration, but the threshold concentration and yield decrease vary with the crop species and cultivar (Ponnamperuma, 1994). Rice is considered to be moderately sensitive to salinity. The degree of salt injury reportedly depends on the salt concentration, pH, temperature, air humidity, solar radiation, water depth, duration of exposure, and the crop growth stage (Levitt, 1980; Akbar and Ponnamperuma, 1982). Vegetative growth of rice in salt-affected soils is generally better during the wet than during the dry season, mainly the result of a lower vapor pressure deficit and, hence, reduced transpiration rates. Most rice cultivars are severely injured in flooded soils at 8-10 dS m^{-1}. Sensitive cultivars suffer damage already at 2 dS m^{-1}. Salt injury is usually less severe in neutral and alkaline soils than in acid soils, and less severe at 20°C than at 35°C. Rice, which is tolerant during germination, can become highly sensitive during the early seedling stage. Cultivars with a high level of salt tolerance during the vegetative growth can become temporarily salt-sensitive during pollination and fertilization before again increasing the tolerance level at maturity. Salinity during the reproductive stage depresses grain yield much more

than salinity during the vegetative growth stage (Akbar and Ponnamperuma, 1982; Castillo *et al.*, 2003). Typical symptoms of salt injury in rice are stunted growth, leaf rolling, white leaf tip, white blotches in the leaf blade, drying of the older leaves, and poor root growth (Ponnamperuma and Bandyopadhya, 1980).

In addition, salinity can decrease leaf protein concentrations and increase membrane permeability and malonedialdehyde synthesis due to lipid peroxidation. Such an acceleration of deteriorative processes affects all leaves in rice salt-sensitive cultivars while it appears more marked in older than younger leaves of salt-resistant genotypes (Lutts *et al.*, 1996b). Salinity stress during the vegetative stage and at panicle initiation of rice was found to delay flowering and prolong the crop growth duration by five to ten days. This growth duration increase prolongs the period of active photosynthesis, and can in some instances partially compensate for reduced assimilation during the period of salinity stress. Therefore, stress at the transplanting stage was found to have the least effect on biomass accumulation and yield of rice. However, leaf death and tillers abortion caused by severe stress (> 8 dS m^{-1}) at transplanting can not be compensated for and generally results in yield reduction. Salinity of 12 dS m^{-1} and 18 dS m^{-1} imposed at panicle initiation was found to hinder the formation of panicles and spikelets, thus creating a sink limitation, even after the relief of stress. Reported grain yield reductions range from 20-65%, primarily because of the reduced number of filled spikelets. Straw biomass accumulation, however, is generally less affected due to the development of late nodal tillers. High salinity levels (12 and 18 dS m^{-1}) after flowering have the effect of reducing grain weight, grain and total biomass because of the reduced duration of the grain filling phase. The responses of the rice plant to short duration salinity stress were found to be very similar to response to drought. This is because salinity reduced the osmotic potentials of the soil solution. High sodium uptake in treatments with higher reduction in yield suggested that the yield reduction might have been caused by sodium toxicity (Castillo *et al.*, 2003).

1.3. Mechanism of salinity tolerance

Plant survival depends on maintaining a positive turgor, which is indispensable for expansion growth of cell and stomatal opening. A decrease in water availability under soil salinity causes osmotic stress, which leads to decreased turgor. Osmotic adjustment is one of the vital cellular tolerance processes to osmotic stress, conserved in both halophytic and glycophytic plants. Osmotic stress may induce ion (Na^+ and K^+) uptake and compartmentalization into the vacuole, and synthesis of organic compatible solutes such as proline, betaine, polyols and soluble sugars (Shimose, 1995). Use of ions for osmotic adjustment may be energetically more favorable than organic osmolyte biosynthesis under stress, since ion uptake and sequestration into the vacuole may cost only 3-4 moles of ATP compared with the 30-50 moles of ATP needed for the synthesis of one mole organic osmolyte (Raven, 1985).

Salinity causes a drastic decrease in potassium content of salt-sensitive rice varieties, the salt susceptible rice cultivars have the low ratio of K/Na_{leaves} and high grain yield reduction under salinity (Asch *et al*., 2000). Polyamine, putrescine, spermidine and spermine concentrations are reported in term of osmotic shock and desiccation symptoms (Bui and Nguyen, 2004). The salt-overly-sensitive *Arabidopsis* was found to accumulate more proline than wild type plants under salt stress conditions (Liu and Zhu, 1997). An important mechanism is osmotic adjustment, the change in osmotic potential due to accumulation of compatible solutes such as glycinbetain, proline, polyols and sucrose within the cell (Tester and Davenport, 2003). Osmotic adjustment is not only related to grain yield, but also due to an internal physiological consistency. It influences turgor at given potential and influences root growth, delays leaf area loss, increases dry matter and yield (Fischer, 1996). Salt-tolerant cultivars of rice accumulate less Na, Cl, Zn and proline and more K at root and shoot levels than salt-sensitive varieties. P transport from root to shoot was inhibited in the salt-sensitive cultivars. Accumulation of Na, Cl, and decrease in K content at the shoot level were restricted to the oldest leaves in salt-tolerant genotypes while proline accumulated in the youngest leaves in all cultivars. In presence of salinity, the osmotic potentials

of the roots and of the oldest and youngest leaves were lower in the salt-tolerant than in the salt-sensitive genotypes (Lutts *et al.*, 1996a).

Mechanisms to minimize damage from high salinity vary among plants, and several mechanisms must operate in a coordinated fashion to manage Na^+. For example, plants can: (1) minimize initial entry; (2) maximizes efflux; (3) minimize loading to the xylem or maximize retrieval before reaching the shoot; (4) maximize recirculation out of the shoot in the phloem; (5) maximize intracellular compartmentalization or allocation to particular parts of the shoot (e.g. pith cell or old leaves); or even (6) secrete salt onto the surface of the leaf (Tester and Davenport, 2003).

Cellular ion homeostasis under salinity is achieved by following strategies: 1) exclusion of Na^+ from the cell by plasma membrane bound Na^+/H^+ antiporters or by limiting the Na^+ entry, 2) utilization of Na^+ or osmotic adjustment by compartment of Na^+ into vacuole through tonoplast Na^+/H^+ antiporters, and 3) Na^+ secretion (Zhu, 2002).

1.4. Strategies to manage salt stress in the Mekong Delta

As above information, salt stress has serious effects on cultivation. However, in ancient times the coastal areas with flooded and high salinity, only mangrove and *Melaleuca* spp. can survive. Such forests provide the natural barrier against the storm and sea tidal, which destroy crops and erode soil (Nguyen *et al.*, 1998).

In the rainfed and flooded saline affected area, lowland rice is a better crop for farming because it can adapt better to flooded conditions. Farmers selected the adapted cultivars and designed the appropriate cropping season for cultivating in order to reduce the damage of salt stress. Therefore, cultivation activities generally occurred during the rainy season to avoid the lack of fresh water and the effects of saline water intrusion in the dry season. However, in recent years, with the green evolution and progress of technology, the irrigated and water control systems were established as well as the achievement of plant breeding, chemical supply, etc. So, rice production can be carried out the whole year.

Cultural Practices

Recently, in the rainfed and saline affected areas, the appropriate cropping calendar, which is used to minimize the effects of salinity, is single or double rice production characterized by short growth duration in the rainy season from May to December, and cash crops such as watermelon, cucumber, sweet potato, beans, etc. in the early dry season from November to February (Le, 1999). Other farming models like rice-shrimp farming system became popular in recent years. Here, farmers have an adapted strategy to the fluctuating freshwater-brackish water environment by evolving a rice-shrimp rotation system to maximize economic returns through both rice and high-value, extensive or semi-intensive shrimp production (Vo-Tong, 1993; Tran, 1994).

Water management system

Previously, the water management system such as waterways, dikes and water gates were built for irrigation, seawater and flood management as well as transportation. However, the intensity of water management system building was increased in recent years from 1990s (Torell and Salamanca, 2003), in order to reclaim the unfavorable areas such as saline affected and rainfed areas, or acidic and deep flooded areas for enhancing rice production.

Tolerant rice genotypes

Before the period of building up water management system (1990s), traditional rice cultivars were dominantly cultivated because they are well adapted to the prevailing saline and flooded conditions. However, these cultivars only grow once a year because most of them are sensitive to photoperiod (heading from November to December, short-day conditions). They have growth durations of more than 6 months, a low yield potential (3 ton ha^{-1}) and are frequently sensitive to pests and diseases. Such traditional cultivars, which are however better adapted to saline conditions than most modern semi-dwarfs include Doc Do, Doc Phung, Nang Co Do, Nang Keo, Tai Nguyen, Tep Hanh, Mot Bui, etc. (Le, 1999). In recent years,

with rapid demographic growth and the development of a water management system, traditional cultivars have been increasingly replaced by high-yielding, short-cycled semi dwarf cultivars allowing for the cultivation of up to three irrigated crops per year. Today, triple rice cropping is widespread in the Mekong Delta with crop yields of up to 6 ton ha^{-1} and crop and mean growth durations of 85-110 days. In the progress of rice breeding, some short-cycled genotypes, which moderately tolerate saline conditions, e.g. MTL119, OM1314, OM1490, OM2031, were selected and released (Nguyen *et al.*, 2003; Bui and Nguyen, 2004; Nguyen and Nguyen, 2004).

In summary, salinity is one of the main constraints for rice production in the Mekong Delta. While farmers and scientists have managed to reduce crop damages due to salt stress, above-mentioned strategies show a number of disadvantages. For example, most salt tolerant cultivars have low grain quality, or long growth duration (IR42, Pokali, etc.); moderately tolerant cultivars are often severely damaged if saltwater intrudes before the ripening stage of the crop or when the salt concentration exceeds 0.3% (5 dS m^{-1}) (Akbar, 1982). To successfully overcome the effects of salt-stress, we hypothesize that a combination of several strategies is required. Besides the cultural practices mentioned new strategies are now available. These are based on the recognition of brassinosteroids, plants derived phytohormones, that can reportedly enhance the tolerance of a range of crops against both biotic and abiotic stresses. The possible effect of these brassinosteroids on the salt tolerance of rice has not been investigated.

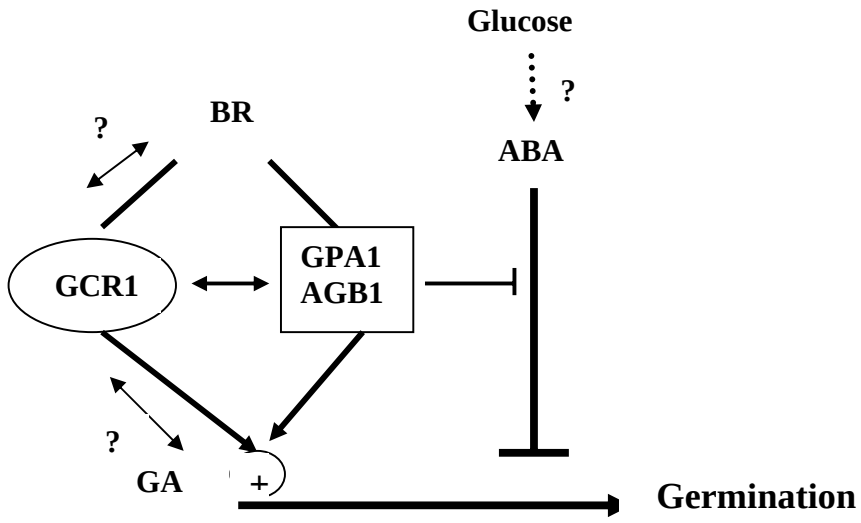
1.5. Brassinosteroids and stress tolerance

Brassinosteroids (BRs) are naturally occurring steroidal plant growth regulators. Since Grove reported that organic extracts of *Brassica napus* L. pollen with structure of brassinolide, which had been collected by bee, promoted stem elongation and cell division in plants (Grove *et al.*, 1979), up to date more than forty related compounds have been found and identified in plants (Yokota, 1997). Among the naturally occurring brassinosteroids, brassinolide and castasterone are considered to be the most important because of their wide distribution, as well as their potent biological activity. BRs have been found in a wide range of plants, including dicots, monocots, gymnosperms and algae, and in various plant parts such as pollen, leaves, flowers, seeds, shoots, galls and stems (Wang *et al.*, 1993). Levels of endogenous BRs vary among plant tissues, pollen and immature seeds are the richest sources with ranges between 1-100 ng g^{-1} fresh weight while shoots and leaves usually have lower amounts of 0.01-0.1 ng g^{-1} fresh weight (Takatsuto, 1994). Several trials demonstrated that treatment of plants with exogenous BRs at appropriate stage of their development results in increase of crop yield and quality (Yokota, 1997; Khripach *et al.*, 2000). Interestingly, plants treated with BRs acquired resistance or tolerance against a wide variety of stresses including cold, heat, drought, salt, disease, insect and herbicide (Mandava, 1988; Ikekawa and Zhao, 1991; Richter and Koolman, 1991; Clouse and Sasse, 1998; Kamuro and Takatsuto, 1999; Khripach *et al.*, 2000; Roth *et al.*, 2000; Schnabl *et al.,* 2001; Anuradha and Rao, 2001; Abdullahi *et al.*, 2003; Anuradha and Rao, 2003; Krishna, 2003; Vardhini and Rao, 2003; Singh and Shono, 2005).

1.5.1. Physiological responses to brassinosteroids

Brassinosteroids promote seed germination and rescue the germination phenotype of gibberellin biosynthesis mutants of *Arabidopsis thiana* (Steber and McCourt, 2001; Chen *et al.*, 2004). Gibberellins and light seem to act in common pathway to release photo-dormancy, whereas BRs do not release photo-dormancy (Leubner-Metzger, 2001). Brassinosterios induced seed germination of rice (Anuradha and

Rao, 2001), tobacco (Leubner-Metzger, 2001), and *Eucalyptus camaldulensis* (Clouse and Sasse, 1998).



GCR1 (G protein ɣ-subunit), GPA1 (G-protein α-subunit), AGB1 (G-protein ß-subunit). ABA (abscisic acid), GA (Gibberellic acid)

Figure 3. Schemes of GCR1 modes of action in Arabidopsis seed

GCR1 encodes a protein with predicted seven-transmembrane spanning domains and other features characteristic of seven-transmembrane receptors. GCR1 positively regulates seed germination, by coupling or modulating BR potentiation of GA-stimulated germination, but acts in a pathway independent of GPA1 and AGB1. GPA1 and AGB1 also negatively regulate the antagonistic effect of ABA on GA-stimulated germination. Glucose delays seed germination, but not necessarily via ABA (adapted from Chen *et al.*, 2004).

Cell expansion and division

BRs were found to have high effectiveness on elongation, bending and cell division (Clouse and Sasse, 1998). BR application at low concentration (nmol to µmol level) promotes elongation of hypocotyls, epicotyls, and peduncles of dicots, as well as coleoptiles and mesocotyls of monocots (Clouse, 1996). Both BR and auxin induce elongation but their kinetics are quite different. Auxin generally shows a very short lag time of 10 to 15 minutes between application and the onset of elongation, with maximum rates of elongation reached within 30-45 minutes. Conversely, BR has a lag time of at least 45 minutes with elongation rates continuing to reach several hours (Zurek *et al.*, 1994). However, the inhibitory effects of BRs on expansion have been widely reported in root tissue. In general, exogenous application of BRs inhibits primary root extension and lateral root formation, with occasional promotions of elongation or adventitious rooting seen with less than picomol concentration (Clouse *et al.*, 1993). Inhibitory effects, particularly on expansion, are often mediated via the induction of ethylene biosynthesis, and treatments with exogenous BRs increase the production of ethylene in stem tissue (Wang *et al.*, 1993).

In cultured parenchyma cells of *Helianthus tuberosus*, application of nanomolar concentration of BR stimulated cell division at least 50% in the present of auxin and cytokinin (Clouse and Zurek, 1991). In Chinese cabbage protoplasts, 24-epibrassinolide, when applied with 2,4-D and kinetin, promoted cell division in a dose-dependent manner and enhanced cluster and colony formation (Clouse and Sasse, 1998). Like cytokinins, BRs have also been reported to be involved on branching responses. BRs also have been demonstrated to change endogenous cytokinin levels in various plant species (Pereira-Netto *et al.*, 2003).

Vascular differentiation

Auxin and cytokinin are required for the initiation of xylem development both *in vivo* and *in vitro* (Fukuda, 1997). This evidence demonstrated that BRs have a significant role in vascular differentiation. In *Helianthus tuberosus*, one of the major *in vivo* systems for studying xylem differentiation, nanomolar concentration of

exogenous brassinolide increased the differentiation of tracheary elements up to 10-fold after 24h. Normally, tracheary element differentiation requires at least 72h in this differentiation process (Clouse and Zurek, 1991).

Reproduction

Pollen is a rich resource of endogenous BRs and *in vivo* studies have suggested that pollen tube elongation could depend in part on BRs (Clouse and Sasse, 1998). The effects of BRs on sexual differentiation in plants are shown by induction of bisexual and pistillate flowers (Suge, 1986).

Modulation of stress responses

BRs can induce plants resistance to environmental stresses such as drought, salt, low or high temperatures, etc. The enhanced resistance was attributed to BR-induced effects on membrane stability and osmoregulation (Wang *et al.*, 1993). In rice, 24-epibrassinolide treatment reduced electrolyte leakage during chilling at 1-5°C, reduced malonedialdehyde content, slowed the decrease in activity of superoxide dismutase, but enhanced levels of available ATP and proline (Clouse and Sasse, 1998). Tomato plants treated with 24-epibrassinolide enhanced tolerant to high temperature, induces expression of mitochondrial small heat shock proteins, which possibly induces the thermotolerance (Singh and Shono, 2005). BRs induced salt tolerance in rice (Anuradha and Rao, 2001; Anuradha and Rao, 2003), 24-epibrassinolide protected the leaf cell ultrastructure of cereal leaves under salt stress and also prevented nuclei and protoplast degradation (Wang *et al.*, 1993). BRs improve drought stress in sugar beet and wheat (Sairam, 1994). BRs enhanced resistance to several stresses, such as cold, salt, fungal infection, herbicide injury (Hamada, 1986; Takematsu *et al.*, 1986; Mandava, 1988; Clouse and Sasse, 1998), and insect infestation (Richter and Koolman, 1991).

Brassinosteroids remove the inhibitory effect of salt stress on pigment levels and this could be one of the reasons for growth stimulation by brassinosteroids under saline conditions. The total chlorophyll content is increased in the leaves of wheat (Sairam, 1994), rice (Wang *et al.*, 1993), *Brassica juncea* and *Vigna radiate*

(Fariduddin *et al.*, 2003). Brassinosteroid had an important role in membrane stability and osmoregulation (Rao *et al.*, 2002). Proline content in EBl treated mung bean hypocotyls segments was remarkably enhanced under stress conditions (Zhao and Chen, 2003).

1.5.2. Practical applications of brassinosteroids

The yield of crops is directly influenced by germination, rooting rate and ratio of seed or fruit set. These yield components greatly depend on the environmental conditions such as temperature, drought, salt, flood, etc. as well as fungal infection or insect infestation. The stability and improvement of agricultural production can be achieved by BRs application (Kamuro and Takatsuto, 1999).

BRs have been confirmed to enhance salt tolerance in rice under 50 mM NaCl conditions (Hamada, 1986), seed treatment with dilute solution of BRs considerably improved the growth of rice plants in saline media, (Rao *et al.*, 2002). 24-epibrassinolide and 28-homobrassinolide reversed the inhibition of germination and seedling growth rates of rice under salinity stress and enhances levels of nucleic acids and soluble protein synthesis (Anuradha and Rao, 2001). The 28-homobrassinolide and 24-epibrassinolide are very effective in increasing the percentage of germination and seedling growth rate of sorghum under osmotic stress. The growth promotion was associated with enhanced levels of soluble proteins and free proline content (Vardhini and Rao, 2003). An enhancement of the length and number of roots of rice plants cultivated under salt stress conditions from seed soaked in a homobrassinolide solution was reported (Ueono *et al.*, 1985; Takematsu and Takeuchi, 1989). Rice seeds were soaked in a solution of BRs before germination, root weight and rooting ability were significantly increased also when rice plants at tillering were fed by BRs through the roots or by foliar spray treatment (Wang *et al.*, 1993). Brassinolide is known to increase the number, size and grain weight as well as the ripening rate (Hirai, 1991; Krishnan *et al.*, 1999). It increases both translocation of assimilates and the accumulation of starch in the panicle (Fujii and Saka, 1992). Brassinolide is assumed to increase the sink capacity of panicles after heading by promoting the accumulation of starch in the

panicle, resulting in promotion of the panicle ripening (Fujii and Saka, 2001). Besides increasing the germination rate, promoting growth and development in normal and stress conditions, BRs treatment enhances cereals resistance to lodging (Prusakova *et al.*, 1985).

"COM-CAT-powder", a natural plant extract from seeds of *Lychnis viscaria* (a product of Polus, Maxiplant GmbH, Lindenfels, Germany), which contains 24-epicastasteron and 24-episecasteron, was applied in field trials. It increased the yield of different crop cultivars in various countries. For example, "COM-CAT-powder" increases the yield of wheat 5.7-19.3%, maize 5.4-12.8%, and the other crops such as potatoes, soybeans, cabbage, carrots, onions, cucumber, strawberries and eggplants (data given by Polus, Maxiplant GmbH, Lindenfels, Germany). The yield increase depends on the soil and climatic conditions (Friebe *et al.,* 1999; Schnabl *et al*., 2000).

BRs application highly depends on mode and time of treatment (Anon, 2002). When two modes of treatment were compared, spraying the seedlings was found to be more efficient than seed soaking in respect on synchrony of shoot development, shoot forming capacity and productivity. Usual mode of BRs application in agriculture consists in soaking seeds in an appropriate solution or spraying young plants. Results of a foliar spray method were found to be highly dependent on the phase of plant development, as well as the concentration of solution. Generally, better results can be obtained when young rather than old plants are treated. The formulation of the spraying solution is very important, and additives are necessary to facilitate the spreading of the active substance, in order to prevent early drying and to ensure penetration of BRs via the cell walls (Khripach *et al.*, 2000). The point of time for spray application is an important factor and may account for the inefficiency of treatment using naturally occurring BRs which appear to be rapidly degraded within the plant (Kamuro and Takatsuto, 1999). Besides, addition of EBI to fertilizers with prolonged period of action allows increasing crop yield and improving quality of several crops, this application mode additionally minimizes human labor (Khripach *et al.*, 2000). A different approach has been elaborated and offered for the use (Pirogovskaya *et al.*, 1996).

BRs application highly depends on cultivars, climatic conditions, type of soil, level of applied fertilizers, and others. For example, in many field trials, the effect of BRs on rice differed greatly at different cultivation temperatures and also light conditions such as day length and spectral quality, therefore the trial results in different seasons are not always the same, even employing the same crop (Kamuro and Takatsuto, 1999). In the early 1980's scientists of United States Department of Agriculture showed that BRs could increase yields of radish, lettuce, bean, pepper and potatoes in some preliminary trials. However, subsequent results under field conditions were disappointing because inconsistent results were obtained. More recently, large scale field trials in China, and Japan over a six year period have shown that 24-epibrassinolide, an alternative to brassinolide, increased the production of agronomic and horticultural crops including wheat, corn, tobacco, watermelon and cucumber. But also here the results depend on cultural conditions, method of application, and other factors (Arteca, 1995).

Generally, BRs have wide spectrum effects on diverse plants. However, the results of BRs application differ greatly and are highly dependent on several factors (as above). Therefore, before widespread application for cultivars is achieved, the effects of BRs on these cultivars, which have been grown in its environmental conditions, have to be investigated elaborately.

1.6. Hypothesis and Objectives

In Japan, Korea and China, rice seed treatment with BRs increased the yields and tolerance against various stresses (Yokota, 1997). Especially, when crops were grown in more stressful conditions, BRs application showed highly significant effect, so BRs were called “stress hormone” (Castle *et al.*, 2003). With current cultural practices being insufficient to effectively address the diversity of salinity conditions and environments and former inability to adopt such measures, the application of BRs may provide a promising new strategy to address the salinity problematic of the Mekong Delta. The present study involved the investigation of the effects of 24-epibrassinolide (EBl) application on the salt tolerance in rice are based on the following objectives:

- Effects of an application of the brassinosteroid “EBI” on the germination of rice under saline conditions
- Effects of EBl application on the salinity tolerance of two rice cultivars during the seedling stage
- Effects of EBl application on the salinity tolerance of two rice cultivars during the vegetative growth stage
- Effects of EBl application on the salinity tolerance of two rice cultivars during the reproductive stage
- Investigation of possible mechanisms of EBI-induced salinity tolerance

2. MATERIALS AND METHODS

2.1. EXPERIMENTAL CONDITIONS

2.1.1. Experimental site

Experiments were carried out in Center of Advanced European Studies and Research (CAESAR) from June 2003 to March 2006 in growth chamber and greenhouse conditions.

2.1.2. Growth chamber conditions

Climatic conditions in growth chamber:

- 28^{o}C temperature
- 85% humidity
- Illumination: 12h cycles of light and dark, alternately

Figure 2. Rice seedlings grown under growth chamber condition

2.1.3. Greenhouse conditions

Figure 3. Greenhouse conditions with mobile lamp system available in CAESAR

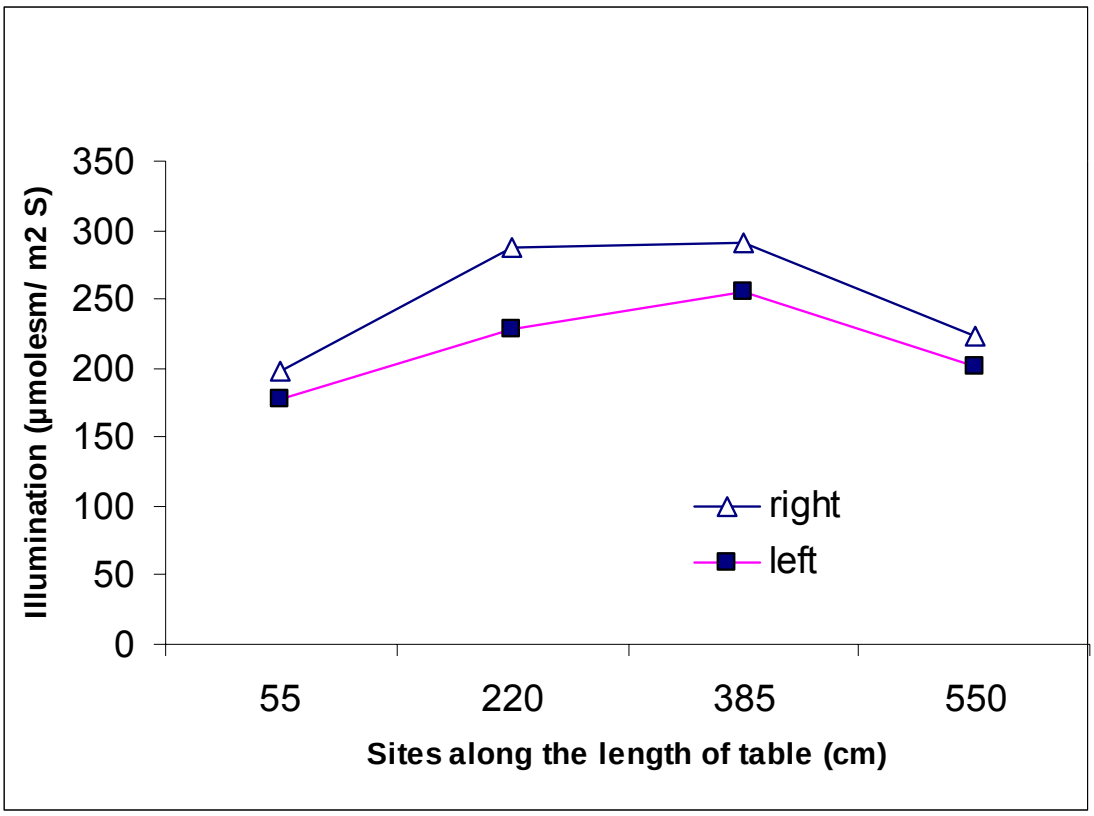
Illumination, temperature and humidity conditions in the greenhouse are presented in Table 2.

Table 2. Climatic conditions in greenhouse

Climatic Factor	Adjusted Conditions	
Light Conditions Illumination	12h light 12h dark	PAR 235 µmol m^{-2} s^{-2}, average Provided by Phillips SON T-Agro 400 Na HP-Lamps switching point 70000lux
Temperature:	30°C	Ventilation temperature 32°C Heating temperature 28°C
Humidity	80%	

Distribution of illumination in the greenhouse

Illumination was measured at the time without sunlight. Therefore, light conditions only affected by lamp system in the green house. Each site along two sides of the length of table had different values of illumination (Fig. 6). The middle of table received the highest light in compared to others positions. On the other hand, in daytime the light conditions were strongly affected by sunlight depending on the position of sun. This result demonstrated that illumination was not equal within the table. Therefore, three blocks of an experiment were designed in a table, in which block 2 in the middle received the highest light density than two other blocks in two edges of table.



right: the right side of table; left, the left side of the table

Figure 4. Illumination distribution in the experimental table of the greenhouse

2.2. GROWTH MEDIA

- Petri dish: using in germination trials, seed were distributed on filter paper (Whatman No.1).

- Nutrient solution: according to Yoshida (1976), using in experiments at seedling stage under growth chamber conditions.

- Experimental soil

Loamy-silt soil from the Meckenheimer Börde, which was supplied by Grafschafter Krautfabrik-Josef Schmitz KG, D-53340 Meckenheim, was used for the experiment. Main available nutrients of soil were analyzed by the Landwirtschaftliche Untersuchungs und Forschungsanstalt (LUFA), and using Standard methods the result is presented in Table 3.

Parameters	Value
pH	7.5
Available P*	82 mg kg^{-1}
Exchangeable K*	257 mg kg^{-1}
Mg	160 mg kg^{-1}

Table 3. Selected characteristics of experimental soil (LUFA) used in the greenhouse experiment

* Extracted with NH_4(Ac-Lac)

Silt-loam soil was sterilized for 4h by Soil Sterilizer (Sterilo) and homogenized before using.

2.3. PLANT MATERIAL

The two indica rice varieties (*Oryza sativa* L.) IR28 and MTL119 from Cantho University (Vietnam) were choosen for all experiments: IR28, which was released by IRRI (International Rice Research Institute), is a salt-stress sensitve variety, while MTL119 is variety, adapted to the saline conditions in the Mekong Delta.

Table 4. Some agronomic characteristics of IR28 and MTL119 evaluated under field conditions of the Mekong Delta, Vietnam

Agronomic characteristics	IR28	MTL119
Growth duration (day)	100	115
Stem height (cm)	100	110
Grain weight (1000 grains g^{-1})	25	29
Yield (Mg ha^{-1})	4.5	5.5
Adaptation to salt	sensitive	tolerant

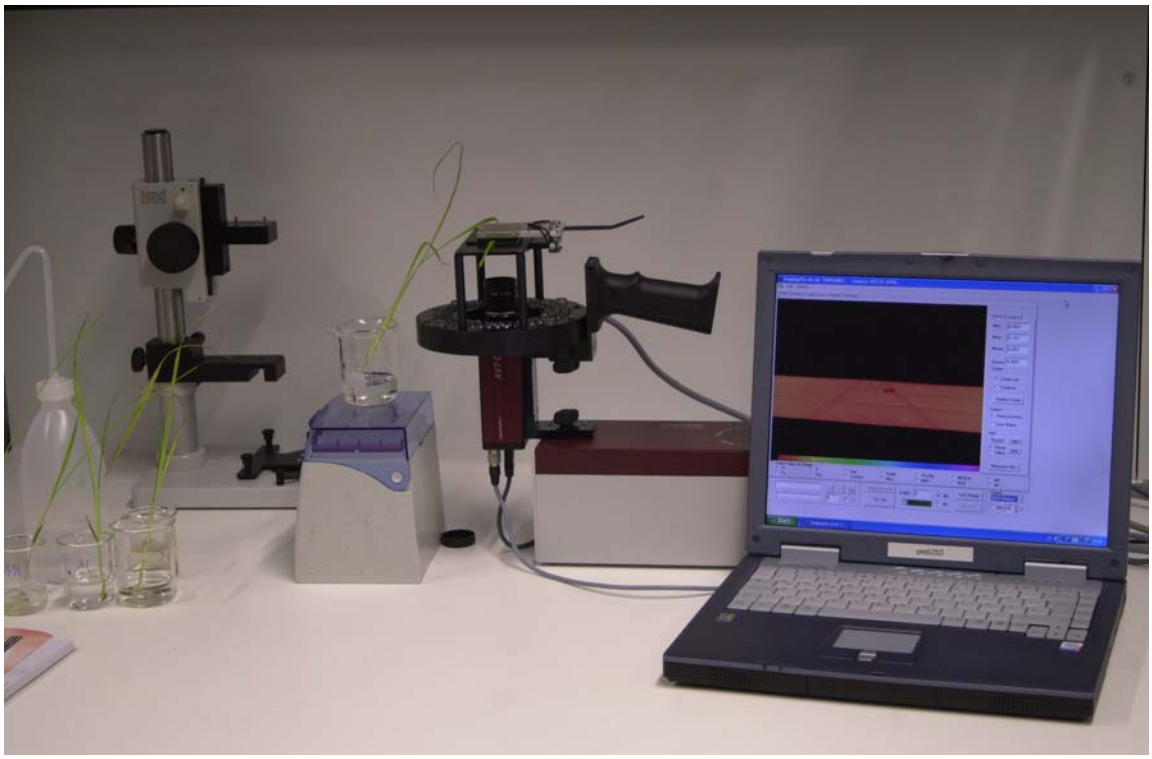


Figure 5. Imaging-PAM system (Walz), this equipment measures photosynthesis of rice leaf based on electron transport and quantum efficiency (yield)

2.4. MEASUREMENTS AND ANALYSES

2.4.1. Morphological parameters

Seeding stage: the stem height, root length, and number of tillers were measured after two weeks experiment in growth chamber conditions.

Vegetative and heading stages: parameters of morphological traits such as plant height, number of tillers were collected at the time of salt stress application, and followed every 10 days until heading.

2.4.2. Physiological parameters

- **The germination rate**: evaluated 5 days after seed imbibition.

- **The healthy seedlings rate**: measured 10 days after seed imbibition.

- **LAI**: was collected at the time of salt stress application, and followed every 10 days until heading. LAI was measured by counting number of leaves per plant, the length and width of smallest, middle and largest leaf and calculated by following formula.

$$LAI = \frac{A * B * C * 0.7}{D}$$

A: number of leaves per plant
B: average of the length of smallest, middle, largest leaves
C: average of the width of smallest, middle, largest leaves
D: area of land per plant, in this case: area of pot per plant
0.7: coefficient for calculating rice leaf area

- **Morphological salinity tolerance:** measured by counting the percentage of injured leaf area per plant.

- **Chlorophyll quantification:** based on the Beer-Lambert Law (Arnon, 1949)

Chlorophyll content was analyzed in order to evaluate the role of EBI in reducing the damage of salt stress on infrastructure of leaf. Chlorophyll can be quantified with a spectrophotometer. Based on the Beer-Lambert Law and the extinction coefficient for chlorophyll, Arnon (1949) devised the following equations for quantification of the total chlorophyll, chlorophyll a, chlorophyll b content in an 80% acetone extract.

Total chlorophyll (μg/ml = 20.2 (A_{645}) + 8.02 (A_{663})

Chlorophyll **a** (μg/ml = 12.7 (A_{663}) – 2.69 (A_{645})

Chlorophyll **b** (μg/ml = 22.9 (A_{645}) – 4.68 (A_{663})

Approximately 0.1 g fresh green leaves (in the middle part) of the 1st or 3rd leaf were homogenized in nitrogen liquid and weighted the powder then put into the centrifuge tube of 2ml and adding 1.5 ml of 80% acetone. Sample tubes were centrifuged at 15,000 rpm for 3 minutes. The supernatant was transferred to a glass cuvette and measured the absorbance at 645 (A_{645}) and 663 nm (A_{663}). Chlorophyll content of leaves was analyzed after 14 days in case of experiments in growth chamber, as well as 1 day before stress and 20 days after stress at tillering stage in case of experiments in greenhouse.

- **Proline assay based on the method of Bates *et al.* (1973).**

Proline accumulation was investigated to assess the salt tolerance of rice by EBI treatment.

Approximately 0.4 g of rice leaves (in the middle part) or roots were homogenized in liquid nitrogen. The resulting powder was weighted and filled into centrifuge tube of 2 ml capacity and 1.8 ml of 3% aqueous sulfosalicylic acid were added. Sample tubes were then centrifuged at 15,000g for 20 minutes. 0.8 ml of supernatants was transferred to test tubes of 10 ml capacitiy and 2ml of acid ninhydrin and 2 ml of

glacial acetic acid were added. Afterwards the sample mixture was heated for 1 hour in boiling water. The reaction was terminated after one hour by putting the test tubes in an ice bath. The reaction mixture was extracted with 2 ml of toluene, mixed vigorously with a vortexer for 15-20 seconds. The chromophore containing toluene solution was aspirated from the aqueous phase, warmed to room temperature and absorbance read at 520 nm using toluene for a blank in glass cüvettes in a photometer. The proline concentration was determined from a standard curve and calculated on a fresh weight basis as follows:

$$P = \frac{y * 2}{115.5 * 0.4 * \frac{1}{2.25}} = \frac{y * 2 * 2.25}{115.5 * 0.4} \quad (\mu mol\ g^{-1})$$

P: proline concentration (µmol g^{-1} fresh weight material); y: proline content (µg ml^{-1})

y = 8,0032 x ^{0,9498} (µg ml^{-1}) (the standard curve)

x: value of optical density was measured at 520 nm

2: content of toluene was used (2 ml); 0.4: fresh weight material was sampled (0.4 g); 115.5: molecular weight of proline

$\frac{1}{2.25} = \frac{0.8}{1.8}$: 0.8 ml of supernatant was taken from 1.8 ml solution of sample and sulfosalicylic acid

Table 5. Time of proline content analysis

Times	Time of analysis	Plant growth stage
1	14 days after sowing	Seedling stage
2	1 day before salt stress/30 days after sowing	Tillering stage
3	10 days after salt stress/40 days after sowing	Tillering stage
4	20 days after salt stress/50 days after sowing	Tillering stage
5	1 days before heading	Heading stage
6	10 days after heading	Heading stage

❖ Gas exchange measurements

Different leaf gas exchange parameters such as CO_2 assimilation (Pn), transpiration (E), stomatal conductance (Gs) were measured with the CIRAS-1 portable infrared gas analyzer equipment. These measurements were carried out on the middle part of the youngest (fully opened leaf) and of the third leaf (from the top) of the main tiller of each plant, 1 day before and 1 day after salt stress as well as 7, 14, and 28 days after salt stress. The measurements were conducted from 9.00 to 12.00 a.m., during this time the curtain of the greenhouse was shut down to avoid effects of different light conditions. The device parameters were set to: leaf surface area 1.75 cm^2, ambient CO_2 concentration (C_{ref}) 100 µmol mol^{-1}, temperature of the leaf chamber varied from 22-25^oC, leaf chamber gas rate (V) 200 ml min^{-1}, photosynthetic photon flux density (PPLD) at the leaf surface was maximum up to 350 µmol m^{-2} s^{-1}.

❖ Electron transport and quantum yield measurements

Photosynthetic electron transport and quantum efficiency were measured in vivo using a portable fluorometer (Imaging PAM). The measurements were carried out

2 days before and 2, 8, 15, 29 days after salt stress, on the youngest, fully opened leaf and on the third leaf of the main tiller of each plant.

$$ET = \left(\frac{Fm' - F}{Fm'} \right) * 200 * 0.84 * 0.5$$

ET: Electron transport rate

F, Fm'= fluorescence under illumination before and after saturating flash

$$QE = \frac{Fm - Fo}{Fm}$$

QE: Quantum efficiency (yield)

Fo, Fm: fluorescence with or without continuous irradiation before and after saturating flash after adaptation in dark before measurement.

2.4.3. Yield and yield components

The grain yield is based on four main components and which were investigated dry matter assimilation and partitioning of plants:

- Number of plants per area unit
- The number of panicles per plant
- The filled grains per panicles,
- The average weight of 1000 grains at 14% humidity of grains

In the conducted experiments, the plant density of all treatments was designed to be 23.5 plants m^{-2}. Therefore, the analysis of grain yield is based on the grain yield per plant. So, only four main yield components, number of panicles per plant, filled grains per panicles, percentage of filled grains per panicle and average weight of 1000 grains had to be measured and analyzed. Besides, percentage of grains per total biomass weight (harvest index) were analyzed to know the carbohydrates partitioning.

- ❖ Filled grains weight per plant (yield)
- ❖ Number of panicles per plant
- ❖ Number of grains per panicle
- ❖ Percentage of the number of filled grains per panicle
- ❖ Weight of 1000 grains
- ❖ Percentage of grains per total biomass weight (harvest index)

$$Yield = \frac{X * Y * Z * 86}{1000 * (100 - A)} \text{ (g/pot)}$$

X: Number of panicles per plant

Y: Number of filled grains per panicle = Number of grains per panicle * percentage of the number of filled grains per panicle

Z: weight of 1000 grains (g)

A: grains moisture

Samples were dried at 45°C for 48h and grain weight was calculated at the standard grains moisture (14%).

2.5. TREATMENT APPLICATION

2.5.1. Growth chamber experiments

Germination test

Rice seeds of the two varieties, the salt-sensitive variety IR28 and the salt-tolerant variety MTL119, were surface sterilized in sodium hypochlorite 0.5% (v/v) for 20', and washed repeatedly in distilled water. Seeds were laid out on filter paper (Whatman No.1) in 9 cm petri dishes (30 seeds per dish). The 24-epibrassinolide (EBl) was added at three different concentrations 0.5, 1.0 and 1.5 ppm (10 ml per petri dish) and with the addition of 0 or 100 mmol NaCl. For the control, 10 ml of methanol at 0.1% were added. Treatments were replicated four times and placed in growth chamber (28°C, 85% humidity and dark condition). Germination was evaluated daily for 5 days. After the 5th day, all seedlings of the treatment with

1ppm EBl and of the untreated control were transferred to potted soil and grown in a greenhouse. After 5 days of growth, seedling growth was evaluated.

Experiment of EBl application on salinity during seedling stage

Homologous seeds of the both rice varieties were soaked for 20 minutes in warm water (54^{o}C) and incubated for 24 hours in 1 ppm 24-epibrassinolide (diluted in 0.1% Methanol), Control seeds were incubated in 0.1 % methanol. The seeds were rinsed with deionized water and placed on filter paper (Whatman No.1) in 9 cm petri dishes for 48 hours. After 48 hours (12h cycles of light and dark, alternately), seedlings were transferred into beakers (650ml), covered by black sheet (for roots growth), with eight seedlings per beaker and filled up with 500ml Yoshida nutrient solution (Fig. 4). Within the first three days, half strength Yoshida nutrient solution was used. Between the 4th and the 7th day the solution was supplemented with 50mmol NaCl in the salinity treatments. During the second week, full strength nutrient solution was supplemented with 100 mmol NaCl (corresponding to about 10 dS m^{-}1) for salt-stress treatment. The experiment was carried out in triplicates in the growth chamber (28^{o}C temperature, 85% humidity). After two weeks, seedling growth were evaluated. Water loss by evapo-transpiration was compensated daily with distilled water.

2.5.2. Greenhouse experiments

After 48h in the dark condition, the pre-treated germinated seeds were transferred into pots, (13 cm in diameter and 14 cm in height with small bottom outlet), which were filled with loam silt soil. Two germinated seeds were sown per pot and placed 3 pots on a floating tray with 3cm level of water. Trays were randomly ranged on tables (1.85 x 5.6 m) with 3 replications and the density was 23.5 pots m^{-2}. Around the tray, buffer plants (MTL119) were used to separate the treated and non-treated trays. The experiments were carried out in climatic greenhouse conditions (Table 2). During the first two days after sowing, the greenhouse illumination was kept off for providing better conditions for root growth of freshly germinated seeds. After

one week, abnormal seedlings (dead, too small or too big) were replaced, and only one normal healthy seedling per pot was maintained.

Water management: fresh water (pH: 7 -7.5, EC: 280 $\mu S\ cm^{-1}$) was maintained about 3cm level in tray and renewed every week.

Fertilizer application: fertilizer NPKS 14-10-20-3 was applied with 0.1g pot^{-1} at 15, 30 days after sowing, and 20 days before heading.

Experimental design

Experiments in the greenhouse were designed by randomized complete block (RCB) in triplicate with experimental factors as Table 6.

Table 6. Factors of experiment

Factor	Factor Level	Factor Value and Characteristics*
Variety	a=2	a_1= IR 28 a_2= MTL 119
Seed and foliar treatment	b=2	b_1= without EBl b_2= with 1ppm EBL
Salt Treatment	c=2	c_1= without NaCl c_2= with 50 or 100mmol NaCl

Brassinosteroid application

Pre-treatment: the treated seeds were soaked into 24-epibrassinolide (EBl) with 1ppm concentration was diluted in 0.1% methanol for 24 h before germination, and the untreated seeds were soaked into 0.1% methanol.

Foliar spraying: 24-epibrassinolide (EBl) at 1ppm was diluted in 0.1% methanol and supplemented with few drops of Tween 20 were sprayed on leaves of the treated plants and the untreated plants were sprayed by methanol 0.1% adding few drops of Tween 20.

Table 7. Time and method of 24-epibrassinolide (EBl) application

Times	Time of application	Method of application
1	Pre- germination of seeds	Soaking seeds into 1 ppm EBl for 24h
2	25 days after sowing, start of tillering	Foliar spraying with 1 ppm EBl (1ml pot^{-1})
3	10 days before heading	Foliar spraying with 1 ppm EBl (2 ml pot^{-1})

Salt stress application in greenhouse experiments

- Experiments for applying salt stress during vegetative stage: salt stress was applied during 30-51 days after sowing with 50 and 100 mmol NaCl (approximately 5-10 dS m^{-1}), the fresh water and salt solution were renewed every week.
- Experiments for applying salt stress during heading stage: salt stress was applied 1 week at heading time starting from 80% of headed plants.

Weed control: weeds were cleaned up manually during the experiment time.

Pest management: aphis and spider mite were biological controlled by natural enemies such as *Aphelinus abdominalis*, and *Phytoseiulus persimilis*, respectively. Chemicals such as pesticide, fungicide and herbicide were not used in this experiment because these chemicals might interact with EBl.

2.6. STATISTICAL ANALYSES

Data were averaged in Excel. Then parameters were analyzed for variance by ANOVA using SPSS version 12.1. The design application was 2 Treatments x 2 Varieties x 2 Salt Stresses factorial. The mean comparison was done by Turkey/DMRT Test.

3. RESULTS

3.1. Effects of 24-epibrassinolide application on germination rate of rice under salt-stress

Germination

Actual effects of 21-epibrassinolide (EBl) treatment on rice seed germination are shown in Table 8. The results indicate that EBl had no significant effect on seed germination of the salt-sensitive variety IR28. After 3 days, the germination rate of both rice cultivars and in all treatments reached 100%. Thus, the germination rate of IR28 was neither affected by EBl nor by salt. However, EBI treatment of MTL119 seeds had a significant effect on seed germination under both non salt-stress and salt-stress conditions (Figure 8). After 4 days, the EBl treatment with a concentration of 1 mg l^{-1} increased seed germination above the non-treated control by 24% and 50% under non salt-stress and salt-stress conditions, respectively. In addition, this effect of EBl on germination was only observed in freshly harvested seeds of cultivar MTL119, while it did not occur in older stored seeds of either cultivar.

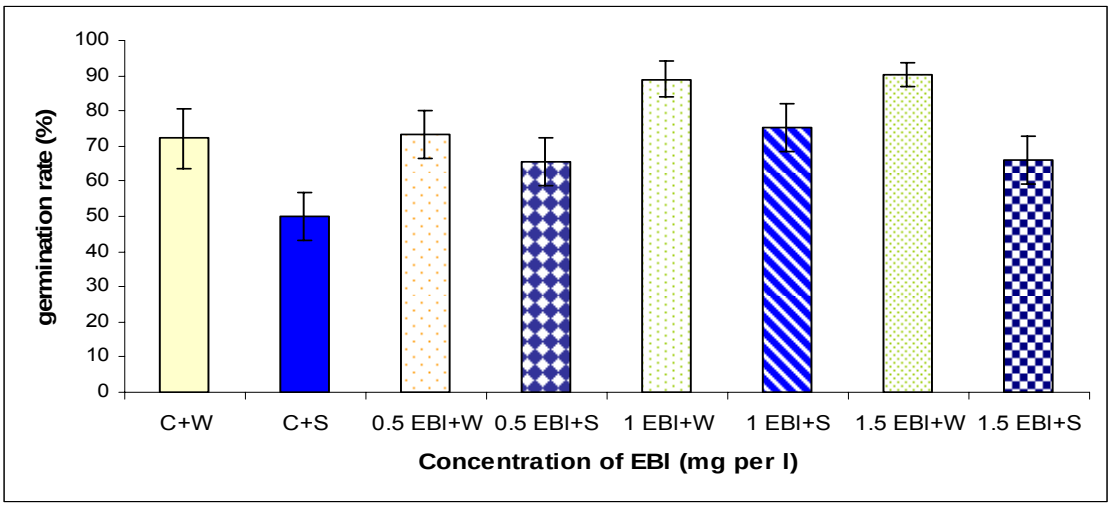
Although MTL119 is known to be salt tolerant, and hence better adapted to salt-stress conditions than IR28, the germination rate of MTL119 was lower than that of IR28 under condition of 100 mmol NaCl concentration (Table 9). Under saline conditions and non-pretreated EBl, the germination rate of MTL119 and IR28 after 5 days was 50% and 100%, respectively. Thus, EBl promotes the germination of rice seeds but it only influences significantly the seed germination of MTL119. Furthermore, at germination stage the salt-sensitive variety IR28 was more tolerant to salt-stress than the salt tolerant variety MTL119. The best concentration of EBl for enhancing germination rate of rice seed was determined to be 1 mg l^{-1}. Concentration of EBl of more than 4 mg l^{-1} inhibited seed germination. Similarly, concentrations of less than 0.5 mg l^{-1}, showed no effect on the germination rate, irrespective of cultivar or salinity treatment.

Table 8. Influence of 24-epibrassinolide application on germination rate of MTL119 and IR28 under non salt-stress and salt-stress (100 mmol NaCl) conditions

Variety	Treatment	Germination rate (%)			
		2days	3days	4days	5 days
IR28	Control+ water	96.	100	100	100 [a]
IR28	Control + salt	55	100	100	100 [a]
IR28	0.5mg l^{-1} EBl + water	98	100	100	100 [a]
IR28	0.5mg l^{-1} EBl + salt	52	100	100	100 [a]
IR28	1mg l^{-1} EBl+ water	99	100	100	100 [a]
IR28	1mg l^{-1} EBl + salt	57	100	100	100 [a]
IR28	1.5mg l^{-1} EBl+ water	100	100	100	100 [a]
IR28	1.5mg l^{-1} EBl + salt	60	100	100	100 [a]
MTL119	Control+ water	26	56	72	72 [bc]
MTL119	Control + salt	6.7	36	50	50 [d]
MTL119	0.5mg l^{-1} EBl + water	51	67	73	73 [bc]
MTL119	0.5mg l^{-1} EBl + salt	16	50	66	66 [bc]
MTL119	1mg l^{-1} EBl+ water	56	80	89	89 [a]
MTL119	1mg l^{-1} EBl + salt	19	62	75	75 [bc]
MTL119	1.5mg l^{-1} EBl+ water	56	90	90	90 [a]
MTL119	1.5mg l^{-1} EBl + salt	17	61	66	66 [bc]
P					<0.01

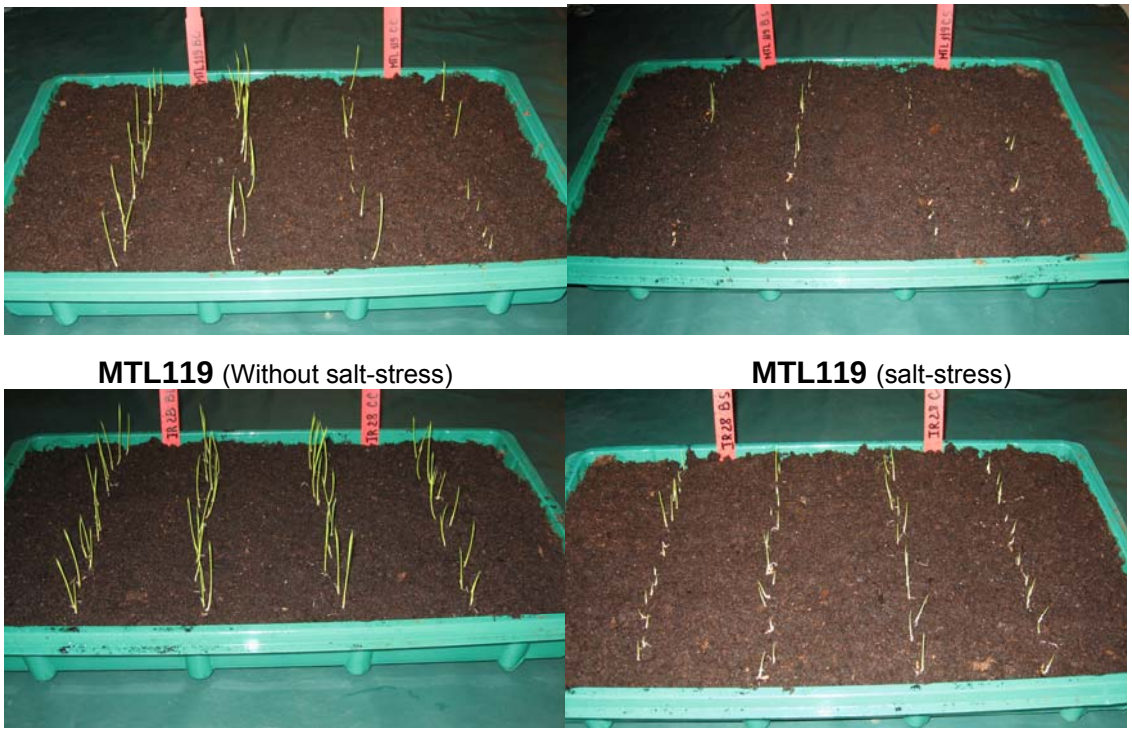
Means in each column with the same letter are not significantly different at P < 0.01

Seeds, one week after harvest, were tested



C: control (without EBI); B : with EBI; S: salt-stress condition; W: water (non salt-stress condition)

Figure 8. Influence of 24-epibrassinolide on germination rate on the salinity tolerant rice cultivar MTL119 five days after imbibition under non-salt stress and 100 mmol NaCl conditions



MTL119 (Without salt-stress) **MTL119** (salt-stress)

IR28 (Without salt-stress) **IR28** (salt-stress)

Figure 9. Influence of 24-epibrassinolide on seedling growth of the salt-tolerant cultivar MTL119 (upper graph) and the salt-sensitive cultivar IR28 (lower graph) ten days after imbibition in soil under greenhouse condition. The two rows on the left of each tray have been treated with EBI at 1 mg l^{-1}.

Seedling quality

Germinated seeds (pretreatment with EBl and salt-stress) were transferred to potted soil, without salt-stress and fertilizer, in the greenhouse conditions (Figure 9). Five days after growing, young seedlings were evaluated. The rate of healthy young seedlings of both varieties was reduced by salt-stress during pre-germination. EBl pre-application did not significantly modify the seedling growth on variety IR28, but it significantly promotes the seedlings growth of MTL119. The EBl pretreated seeds of MTL119 showed higher ratio of healthy plants than of the untreated seeds under both conditions. The EBl pretreatment increased the rate of healthy seedlings up to 85% under normal condition and 129% under salt-stress during pre-germination (Table 9).

Table 9. Influence of 24-epibrassinolide application on the seedling growth of the salt-tolerant variety MTL119 and the salt-sensitive variety IR28 ten days after seed imbibition

Variety	**Pretreatment**	**Healthy seedlings** (%) 10 days after seed imbibition
IR28	Control+ water	100 [a]
IR28	Control + salt	78 [a]
IR28	1mg l^{-1} EBl+ water	100 [a]
IR28	1mg l^{-1} EBl + salt	78 [a]
MTL119	Control+ water	48 [bc]
MTL119	Control + salt	14 [d]
MTL119	1mg l^{-1} EBl+ water	89 [a]
MTL119	1mg l^{-1} EBl + salt	32 [c]
P		< 0.01

Means with the same letter are not significantly different at P<0.01

Root growth

Primary root growth of rice seedling of both varieties was inhibited by EBl pretreated seeds, its shape was curved and its length was shorter than that of the untreated seedlings, which is presented in Figure 10. These results show that 5 days after seed imbibition, the length of primary roots of the EBl pretreated seeds was about 1–2 cm compared to its length of primary roots of the untreated seeds, which accounts for approximately 4–6 cm.

Conversely, secondary root formation was promoted by EBl application. This effect was mostly visible 5 days after seed imbibition. The secondary roots of the treated seeds appeared about 3 days earlier than those of the untreated seeds (Figure 10).

In summary, during the germination period, EBl pretreatment increases the germination rate, promotes secondary root formation and seedling growth. Conversely, it has an inhibiting effect on the primary root elongation, which occurs as the so-called “curved primary root” phenomenon in rice. This phenomenon is a sensible indicator of exogenous applied EBl in rice seedlings.

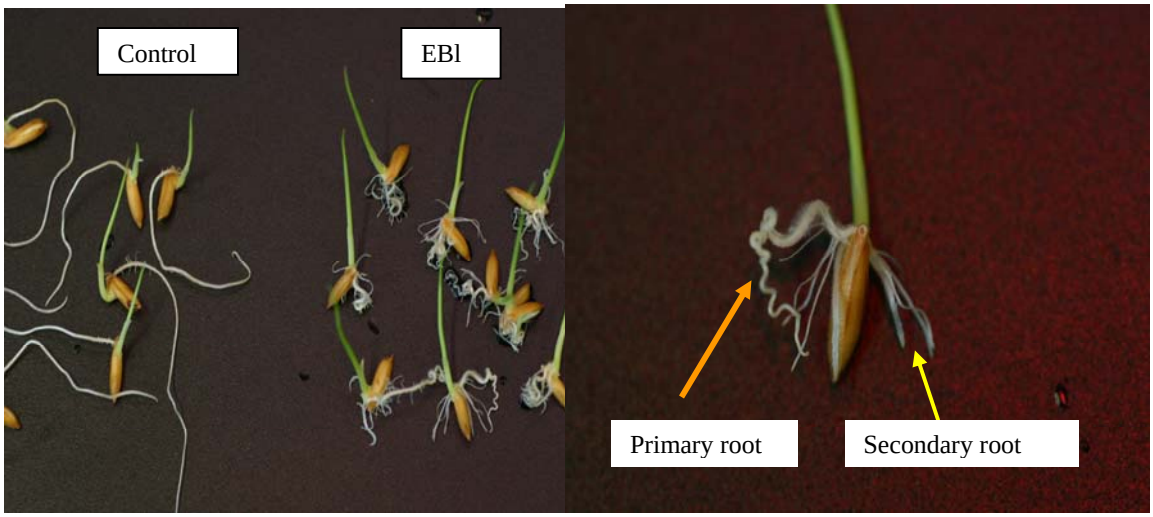


Figure 10. Influence of 24-epibrassinolide application on primary and secondary root of variety IR28 under non salt-stress condition

3.2. Effects of 24-epibrassinolide application on the salinity tolerance of rice during the seedling stage (experiment in hydroponics)

Morphological characteristics

The plant height and root length of the 14 day-old seedlings of both varieties were not significantly affected by EBI application. They were only reduced by salt-stress, which is shown in Table 10.

Table 10. Influence of 24-epibrassinolide application on stem height and root length of 14 day-old seedlings of two varieties MTL119 and IR28 under hydroponics with non salt-stress or salt-stress (100 mmol NaCl) conditions

Variety	Treatment	Stem height	Root length
		(cm)	(cm)
IR28	Control + water	25.83	11.72
IR28	Control + salt	17.11	10.55
IR28	EBI + water	25.89	10.45
IR28	EBI + salt	18.66	9.83
MTL119	Control + water	31.06	11.78
MTL119	Control + salt	24.22	8.72
MTL119	EBI + water	31.89	12.22
MTL119	EBI + salt	24.17	7.11
P		ns	ns

ns: non significant

Chlorophyll

The effect of an EBI treatment on the chlorophyll content of control and salt-affected 2 week-old rice seedlings is presented in Table 11. Neither salt nor EBI application affected the chlorophyll content in either rice variety.

Table 11. Influence of 24-epibrassinolide application on chlorophyll content of 14 day-old seedlings of varieties MTL119 and IR28 under hydroponics with non salt-stress or 100 mmol NaCl conditions

Variety	**Treatment**	**Chlorophyll** (μg g^{-1} fresh leaves)		
		Total chlorophyll	Chlorophyll a	Chlorophyll b
IR28	Control + water	800	607	183
IR28	Control + salt	844	683	151
IR28	EBI + water	671	522	141
IR28	EBI + salt	636	548	80
MTL119	Control + water	515	365	144
MTL119	Control + salt	782	575	198
MTL119	EBI + water	728	556	163
MTL119	EBI + salt	771	584	177
P		ns	ns	ns

ns: non significant

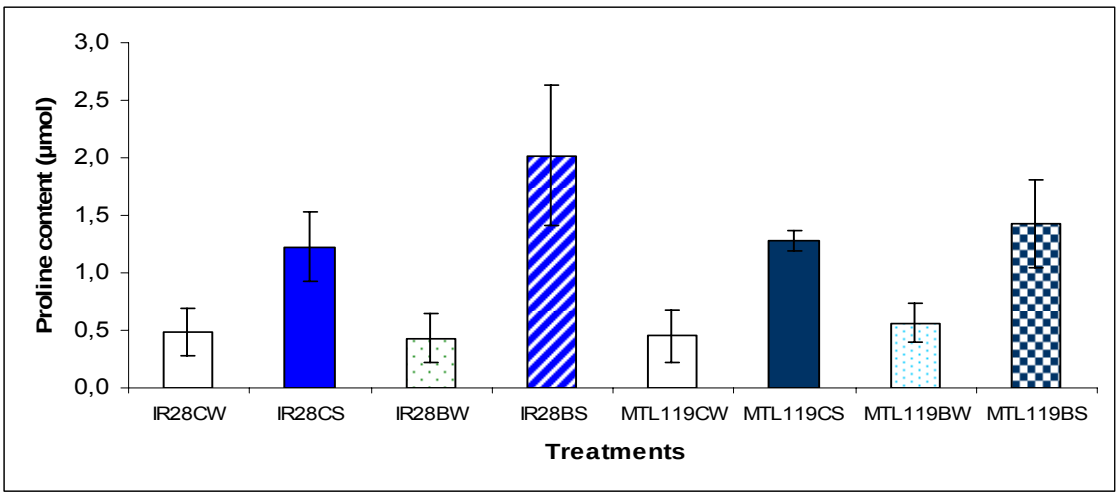
Proline content

The effect of EBI application on the proline accumulation in both leaves and roots of control and salt stressed rice seedlings is presented in Table 12 and Figure 11. Under salt-stressed conditions the proline concentration was higher than in control seedlings. Moreover, the content of proline in leaves of the EBI-treated plants was significantly increased in comparison to the untreated plants ($P<0.01$). Particularly, under salt-stress conditions, the proline content of the EBI-treated seedlings was increased by 65% in variety IR28 and by 12% in variety MTL119. However, the proline content was not affected under non salt-stressed control conditions, irrespective of cultivar or EBI application. In contrast to the leaf, EBI treatment tended to reduce the proline accumulation in the roots of both varieties under salt-stress conditions. It may be concluded that during the seedling stage of salinity affected rice, EBI treatment induces the accumulation of proline in leaves of the salt-sensitive variety IR28, while other parameters (proline content of roots, plant height, root length and chlorophyll content) do not appear to be affected.

Table 12. Influence of 24-epibrassinolide application on proline content of leaves and roots of 14 day-old seedlings of the salt-tolerant variety MTL119 and the salt sensitive variety IR28 under hydroponics with non salt-stress or 100 mmol Nacl conditions

Variety	Treatment	Proline content (µmol g^{-1} fresh weight)	
		Leaves	Roots
IR28	Control + water	0.480 ^{c}	0.115
IR28	Control + salt	1.228 ^{b}	0.340
IR28	EBl + water	0.432 ^{c}	0.1241
IR28	EBl + salt	2.021 ^{a}	0.253
MTL119	Control + water	0.452 ^{c}	0.330
MTL119	Control + salt	1.276 ^{b}	0.422
MTL119	EBl + water	0.563 ^{c}	0.294
MTL119	EBl + salt	1.426 ^{b}	0.338
P		< 0.01	ns

Means in each column with the same letter are not significantly different at P<0.01
ns: non significant



C: control (without EBl); B : with EBl; S: salt-stress condition; W: water (non salt-stress condition)

Figure 11. Influence of 24-epibrassinolide application on the proline content of 14 day-old seedlings of the salt-tolerant variety MTL119 and the salt-sensitive variety IR28 under hydroponics with non salt-stress or 100 mmol NaCl conditions

3.3. Effects of 24-epibrassinolide application on salinity tolerance of rice during the vegetative stage (experiments in potted soil)

3.3.1. Morphological characteristics

Morphological salt tolerance was based on the evaluation of the ratio of injured leaves by discoloured/rolled or dead per plant. An application of EBl reduced the damage of salt-stress during the first thee weeks of stress application (Figure 12). The number of injured leaves of EBI treated plants was always significantly lower than that in untreated plants ($P<0.01$). However, these differences disappeared after 3 weeks.

Under the higher salt concentration (100 mmol), EBl application had no significant effect on morphological salinity tolerance of either variety. The EBl treatment apparently induced some level of rice salt stress tolerance in the tested rice varieties at the tillering stage and this effect persisted until 3 weeks after salt-stress application or 4 weeks after EBl foliar spray.

Plant height and number of tillers were measured every 10 days beginning from the date of salt-stress application (30 days after sowing) until heading. While the plant height was generally reduced by salinity treatments, it was unaffected by an application of EBI between 30 days after sowing (start of salt-stress application) and heading (Table 13). The results in Table 14 indicate that neither the application of a salinity treatment between 30 days after sowing until heading, nor the application of EBI affected the number of tillers in the two rice genotypes.

Table 13. Influence of 24-epibrassinolide application on plant height of rice varieties MTL119 and IR28 under control conditions and with 50 mmol NaCl (upper table) and with 100 mmol NaCl (lower table)

Variety	**Treatment**	**Stem height** (cm)				
	50 mmol NaCl	0 DAS-S	10 DAS-S	20 DAS-S	30 DAS-S	Heading
IR28	Control + water	52	63	80	89	97
IR28	Control + salt	53	65	72	78	89
IR28	EBI + water	51	66	82	91	101
IR28	EBI + salt	50	65	73	78	87
MTL119	Control + water	57	74	84	86	111
MTL119	Control + salt	59	73	77	81	90
MTL119	EBI + water	59	72	85	87	113
MTL119	EBI + salt	60	73	77	82	95
P		ns	ns	ns	ns	ns

Variety	**Treatment**	**Stem height** (cm)				
	100 mmol NaCl	0 DAS-S	10 DAS-S	20 DAS-S	30 DAS-S	Heading
IR28	Control + water	54	65	81	89	100
IR28	Control + salt	51	66	69	72	79
IR28	EBI + water	49	65	80	89	102
IR28	EBI + salt	52	65	71	72	73
MTL119	Control + water	59	72	82	86	113
MTL119	Control + salt	59	72	76	78	88
MTL119	EBI + water	58	71	81	85	110
MTL119	EBI + salt	59	70	76	78	88
P		ns	ns	ns	ns	ns

DAS-S: day after salt-stress; ns: non significant

Table 14. Influence of 24-epibrassinolide application on number of tillers of two varieties MTL119 and IR28 under control conditions and with 50 mmol NaCl (upper table) and 100 mmol NaCl (lower table)

Variety	**Treatment**	**Number of tillers**				
	50 mmol NaCl	0 DAS-S	10 DAS-S	20 DAS-S	30 DAS-S	Heading
IR28	Control + water	3.22	5.81	7.11	7.11	7.11
IR28	Control + salt	3.00	5.36	8.56	8.56	8.56
IR28	EBl + water	3.00	5.73	6.78	6.78	6.78
IR28	EBl + salt	3.22	5.67	8.67	8.67	8.67
MTL119	Control + water	3.00	5.71	7.34	7.53	7.66
MTL119	Control + salt	3.11	5.67	7.21	7.34	7.45
MTL119	EBl + water	3.44	5.41	7.45	7.59	7.78
MTL119	EBl + salt	3.00	6.28	8.03	8.41	9.00
P		ns	ns	ns	ns	ns
Variety	**Treatment**	**Number of tillers**				
	100 mmol Na Cl	0 DAS-S	10 DAS-S	20 DAS-S	30 DAS-S	Heading
IR28	Control + water	3.44	5.55	7.33	7.33	7.33
IR28	Control + salt	3.00	5.66	7.11	7.11	7.11
IR28	EBl + water	3.00	5.56	6.89	6.89	6.89
IR28	EBl + salt	3.00	4.55	7.22	7.22	7.22
MTL119	Control + water	3.22	6.00	7.29	7.53	7.66
MTL119	Control + salt	3.00	5.44	6.67	7.67	7.78
MTL119	EBl + water	3.00	5.89	7.11	7.62	8.11
MTL119	EBl + salt	3.22	5.78	6.78	7.86	8.33
P		ns	ns	ns	ns	ns

DAS-S: day after salt-stress; ns: non significant

3.3.2. Physiological traits

Leaf area index (LAI)

Table 15 shows that the leaf area index (LAI) of both varieties was significantly decreased 30 days after imposing the salt-stress treatment ($P < 0.01$), while LAI was unaffected by EBI application.

Chlorophyll

After one week of salt exposure, depending on genotypes, the top leaves were firstly damaged, the tip of leaf was rolled and discolored then the damage gradually spreads into the old leaves. However, the uninjured leaves of both varieties under salt-stress were visually greener than those under non salt-stress conditions (Figure 13). The analysis the chlorophyll of the old leaves after 21 days exposure to stress was not different between the treated and untreated plants (Table 17). The chlorophyll content of the youngest fully developed leaf of both varieties was significantly increased by EBI treatment, whereas it was seriously decreased under salt-stress conditions. Particularly, the total chlorophyll content of the young leaves of the treated IR28 plants was increased approximately by 100% under both conditions in comparison to control plants. An EBI treatment further increased the chlorophyll content of MTL119 young leaves by 33% and 72% under control and salt-stressed conditions, respectively. In contrast to the seedling stage, the application of EBI did increase the chlorophyll content of rice leaves during the vegetative growth stage, particularly in the salt-sensitive genotype.

Photosynthesis

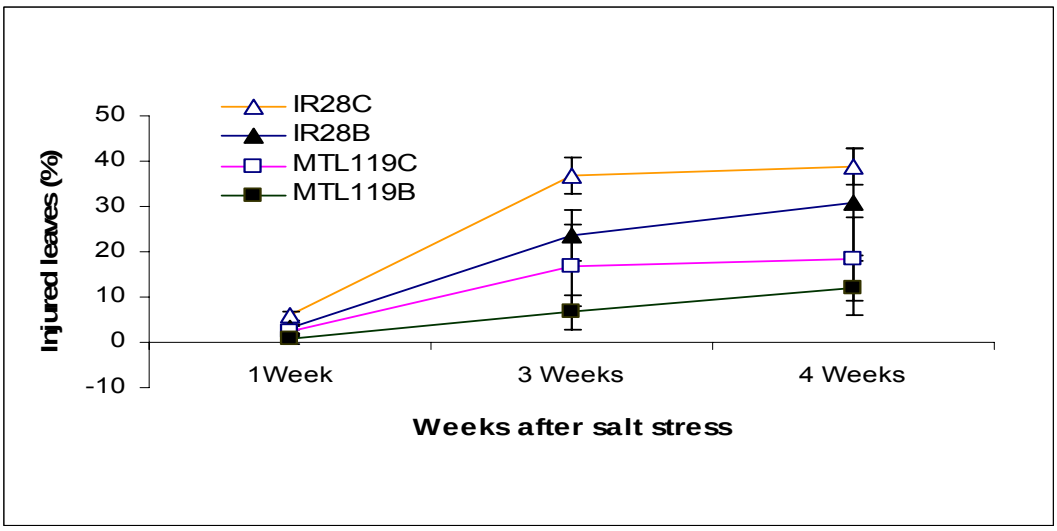
Table 18 indicates that mostly all treatments had negative values of the CO_2 assimilation (Pn) and the values showed large variations. The CO_2 assimilation 16 days after salt-stress was neither significantly different between the EBI treated and untreated plants nor between salt-stress and non-stressed conditions. Although EBI increased the Pn of the treated plants of MTL119 under salt-stress conditions it was not significantly different. The evaporation and the stomatal

conductance (Gs) of both varieties were critically reduced by salt-stress but not statistically affected by EBl.

Table 15. Influence of 24-epibrassinolide application on leaf area index (LAI) of two varieties MTL119 and IR28 control conditions and with 50 mmol NaCl (upper table) and 100 mmol NaCl (lower table)

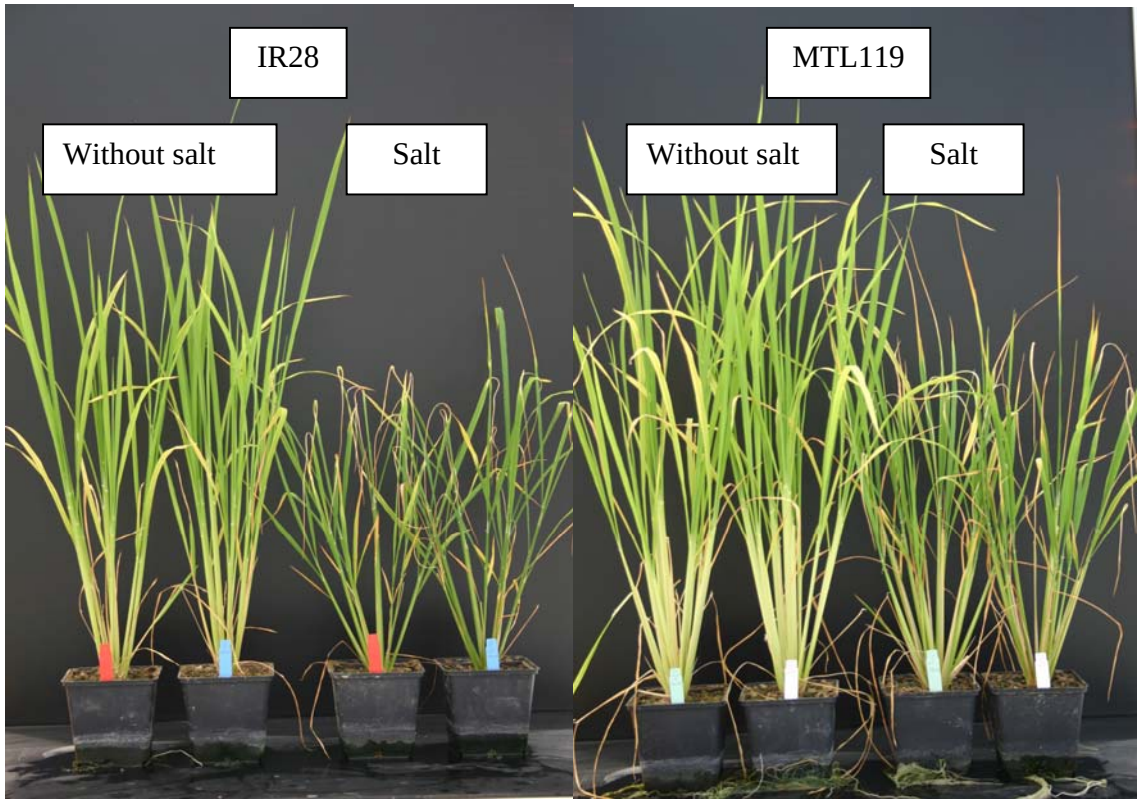
Variety	**Treatment**	**LAI** (leaf area index)				
	50 mmol NaCl	0 DAS-S	10 DAS-S	20 DAS-S	30 DAS-S	Heading
IR28	Control + water	0.99	2.89	5.16	4.35	3.24
IR28	Control + salt	1.01	2.24	4.36	3.54	2.57
IR28	EBl + water	1.05	2.77	4.99	4.46	3.33
IR28	EBl + salt	0.98	2.34	3.85	3.25	2.16
MTL119	Control + water	1.04	2.99	5.11	5.38	3.22
MTL119	Control + salt	1.15	2.35	4.37	4.76	3.10
MTL119	EBl + water	1.09	2.95	4.84	5.31	2.88
MTL119	EBl + salt	1.16	2.54	4.13	4.93	2.75
P		ns	ns	ns	ns	ns
Variety	**Treatment**	**LAI** (leaf area index)				
	100 mmol NaCl	0 DAS-S	10 DAS-S	20 DAS-S	30 DAS-S	Heading
IR28	Control + water	1.10	2.94	5.56	5.17	4.37
IR28	Control + salt	1.19	2.13	3.65	3.11	2.35
IR28	EBl + water	0.95	2.72	4.67	4.29	3.69
IR28	EBl + salt	0.92	1.51	3.20	2.87	1.80
MTL119	Control + water	1.12	2.91	6.25	6.43	3.56
MTL119	Control + salt	1.09	2.18	4.78	5.13	2.30
MTL119	EBl + water	1.16	2.92	5.80	6.36	3.56
MTL119	EBl + salt	1.13	2.22	4.68	5.34	2.43
P		ns	ns	ns	ns	ns

DAS-S: day after salt-stress; ns: non significant



C: control (without EBl); B : with EBl

Figure 12. Influence of 24-epibrassinolide application on morphological salinity tolerance of two varieties MTL119 and IR28 under control and 50 mmol NaCl conditions



The stressed plants are dark green

Figure 13. Influence of salt-stress on 2-month-old plant of IR28 and MTL119 one month after salt-stress application

Table 16. Influence of 24-epibrassinolide application on chlorophyll content of the youngest leaves 23 days after salt-stress of two varieties MTL119 and IR28 under non salt-stress and salt-stress (50 mmol NaCl) conditions

Variety	**Treatment**	**Chlorophyll** (µg g^{-1} fresh leaves)		
		Total chlorophyll	Chlorophyll **a**	Chlorophyll **b**
IR28	Control + water	508 ^{de}	391 ^{de}	111 ^{cd}
IR28	Control + salt	247 ^{f}	187 ^{f}	57 ^{e}
IR28	EBI + water	1039 ^{a}	803 ^{a}	224 ^{a}
IR28	EBI + salt	470 ^{e}	362 ^{de}	103 ^{d}
MTL119	Control + water	595 ^{cde}	455 ^{cde}	134 ^{bcd}
MTL119	Control + salt	411 ^{ef}	314 ^{ef}	93 ^{de}
MTL119	EBI + water	790 ^{bc}	610 ^{bc}	172 ^{b}
MTL119	EBI + salt	707 ^{bcd}	545 ^{bcd}	154 ^{bc}
P		< 0.01	< 0.01	< 0.01

Means in each column with the same letter are not significantly different at P<0.01
ns: non significant

Table 17. Influence of 24-epibrassinolide application on chlorophyll content of the old leaves 21 days after salt-stress of two varieties MTL119 and IR28 under non salt-stress and salt-stress (50 mmol NaCl) conditions

Variety	**Treatment**	**Chlorophyll** (µg g^{-1} fresh leaves)		
		Total chlorophyll	Chlorophyll **a**	Chlorophyll **b**
IR28	Control + water	795	626	160
IR28	Control + salt	1161	907	240
IR28	EBI + water	772	600	163
IR28	EBI + salt	1056	826	217
MTL119	Control + water	1030	794	224
MTL119	Control + salt	1135	884	237
MTL119	EBI + water	963	743	209
MTL119	EBI + salt	834	653	172
P		ns	ns	ns

ns: non significant

Table 18. Influence of 24-epibrassinolide application on CO_2 assimilation of the third leaves 16 days after salt-stress of two varieties MTL119 and IR28 under non salt-stress and salt-stress (100 mmol NaCl)

Variety	Treatment	Photosynthesis		
		CO_2 assimilation ($\mu mol\ m^{-2}\ s^{-1}$)	Evaporation ($mol\ m^{-2}\ s^{-1}$)	Stomatal conductance ($mol\ m^{-2}\ s^{-1}$)
IR28	Control + water	-0.63	4.73	276
IR28	Control + salt	0.61	3.31	165
IR28	EBI + water	-1.10	5.04	295
IR28	EBI + salt	-0.30	3.73	186
MTL119	Control + water	-0.91	4.89	279
MTL119	Control + salt	-0.37	2.63	116
MTL119	EBI + water	-0.76	4.90	275
MTL119	EBI + salt	0.60	2.78	131
P		ns	ns	ns

ns: non significant

Table 19. Influence of 24-epibrassinolide application on electron transport and quantum efficiency of the third leaves CO_2 assimilation of the third leaves 16 days after salt-stress of two varieties MTL119 and IR28 under control and salt-stress (100 mmol NaCl)

Variety	Treatment	Electron transport ($\mu mol\ m^{-1}\ s^{-1}$)		Quantum efficiency (%)	
		1 DAS-S	25 DAS-S	1 DAS-S	25 DAS-S
IR28	Control + water	52.44	35.22	71.9	59.8
IR28	Control + salt	52.72	29.47	72.6	63.9
IR28	EBI + water	50.26	28.02	72.5	60.8
IR28	EBI + salt	52.72	28.36	72.4	56.1
MTL119	Control + water	51.41	29.58	72.4	58.8
MTL119	Control + salt	50.85	30.45	72.6	61.9
MTL119	EBI + water	51.34	35.10	72.4	56.1
MTL119	EBI + salt	49.59	35.41	72.1	59.0
P		ns	ns	ns	ns

DAS-S: days after salt-stress; ns: non significant

The electron transport (ET) and the quantum efficiency (QE) of the two varieties under both salt-stress and non-stressed conditions were not significantly affected by EBl (Table 19). The values of ET and QE were gradually decreased during the tillering period. According to the results of this experiment, EBl had no significant effect on the photosynthesis of the two rice varieties under both conditions during the tillering stage.

Proline content

Table 20 shows that EBl treatment increased proline accumulation of the salt-sensitive variety IR28 up 44% after 12 days salt-stress (DAS-S) with 50 mmol concentration and 17% after 21 DAS-S in comparison to the non-EBl-treated plants. Although EBl treatment enhanced proline accumulation, these increases were not significantly different. Conversely, under control conditions, the proline accumulation of variety IR28 was not affected by EBl application. The proline content of the salt-tolerant variety MTL119 did not differ between treated and untreated plants.

Table 20. Influence of 24-epibrassinolide application on proline content of the third leaves 12 and 21 days after salt-stress of two varieties MTL119 and IR28 under control and 50 mmol NaCl conditions

Variety	Treatment	Proline (µmol g^{-1} fresh leaves)		
		1 DBS-S	12 DAS-S	21 DAS-S
IR28	Control + water	0.097	0.098	0.090
IR28	Control + salt	0.108	0.085	0.145
IR28	EBl + water	0.075	0.094	0.091
IR28	EBl + salt	0.096	0.122	0.169
MTL119	Control + water	0.078	0.088	0.066
MTL119	Control + salt	0.069	0.079	0.071
MTL119	EBl + water	0.070	0.072	0.073
MTL119	EBl + salt	0.070	0.086	0.079
P		ns	ns	ns

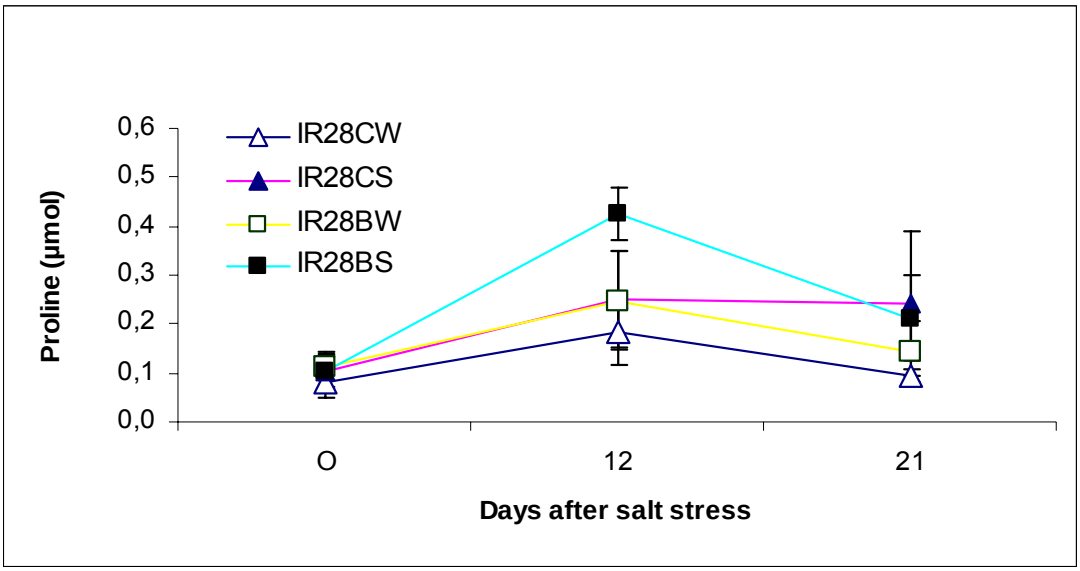
DBS-S: day before salt-stress, DAS-S: day after salt-stress, ns: non significant

At 100 mmol NaCl, an increase of the proline content was observed in all EBI treatments after stress application. Highest proline concentrations were observed 12 days after imposing the salt-stress treatment (DAS-S). Thereafter, the proline content decreased (Table 21 and Figure14). The EBI-treated plants accumulated proline in significantly higher concentrations than in the untreated plants. Particularly, 12 days after salt stress application, the proline content of the treated plants of the salt-sensitive variety IR28 under salt-stress conditions was about 70% higher than that of the untreated plants. However, proline content was not significantly different in the salt-tolerant variety MTL119. Moreover, 21 days after stress proline content were not significantly different between the treated and untreated plants of both varieties (Figure14). During the tillering stage, EBI treatment significantly increased the proline accumulation of the salt-sensitive variety IR28, higher concentrations of applied salt-stress induced higher proline accumulation.

Table 21. Influence of 24-epibrassinolide application on proline content of the third leaves 12 and 21 days after salt-stress of two varieties MTL119 and IR28 under control and 100 mmol NaCl conditions

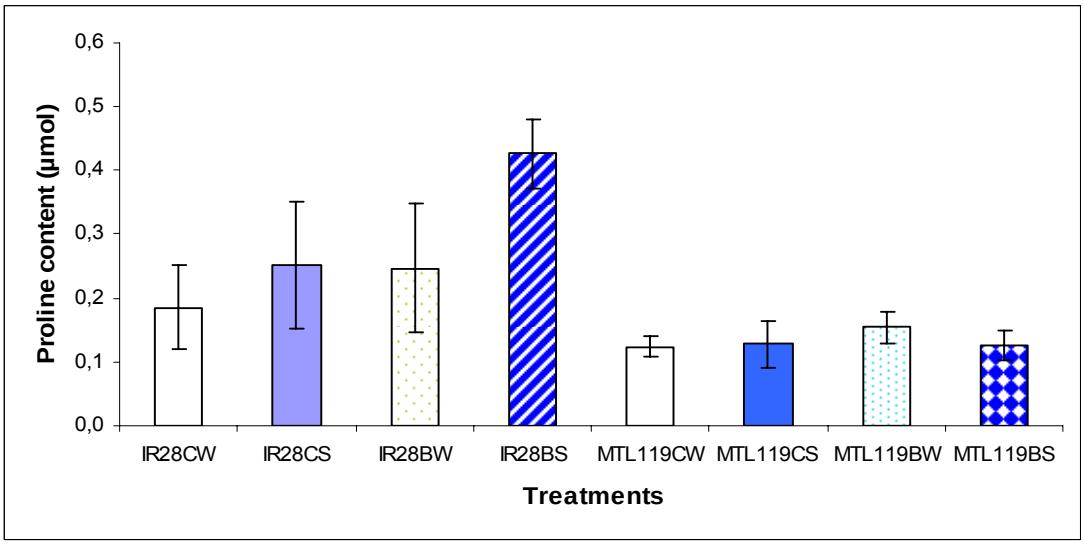
Variety	**Treatment**	**Proline** (µmol g^{-1} fresh leaves)		
		1 DBS-S	12 DAS-S	21 DAS-S
IR28	Control + water	0.080	0.185 ^{cd}	0.095
IR28	Control + salt	0.104	0.252 ^{bc}	0.243
IR28	EBI + water	0.110	0.247 ^{bc}	0.143
IR28	EBI + salt	0.105	0.427 ^{a}	0.211
MTL119	Control + water	0.079	0.124 ^{d}	0.058
MTL119	Control + salt	0.080	0.128 ^{d}	0.103
MTL119	EBI + water	0.076	0.154 ^{d}	0.064
MTL119	EBI + salt	0.076	0.126 ^{d}	0.088
P		ns	< 0.05	ns

DAS-S: day after salt-stress, ns: non significant
Means in each column with the same letter are not significantly different at P<0.05



C: control (without EBI); B : with EBI; S: salt-stress condition; W: water (non salt-stress condition)

Figure 14. Influence of 24-epibrassinolide applications on leaf proline content of the salt-sensitive variety IR28 12 and 21 days after application of 100 mmol NaCl



C: control (without EBI); B : with EBI; S: salt-stress condition; W: water (non salt-stress condition)

Fig 15. Influence of 24-epibrassinolide applications on proline content of the third leaves 12 days after salt-stress of two varieties MTL119 and IR28 under non salt-stress and salt-stress (100 mmol NaCl) conditions

Phenology

Results in Table 22 indicate that the date of heading and harvesting of the two varieties was not affected by EBl applications. However, salt-stress delayed the date of heading and harvesting of MTL119 and IR28 about 7 days and 2 days, respectively. Thus, EBl application appears to have no effect on the phonological development of the two rice genotypes.

Table 22. Influence of 24-epibrassinolide application on the date of heading and harvesting of two varieties MTL119 and IR28 under non salt-stress and salt-stress conditions

Variety	Treatment	Heading date (DAS)		Harvesting date (DAS)	
		50 mmol	100 mmol	50 mmol	100 mmol
IR28	Control + water	79	80	115	116
IR28	Control + salt	81	82	117	118
IR28	EBl + water	79	79	115	115
IR28	EBl + salt	81	82	117	118
MTL119	Control + water	119	120	156	157
MTL119	Control + salt	126	125	163	162
MTL119	EBl + water	117	119	154	156
MTL119	EBl + salt	127	123	164	160

DAS: days after sowing

3.3.3. Grain yield and yield components

Panicle type

Naturally, the primary panicle is headed from the tip node of each mature tiller of the rice plant. Under unfavorable environmental circumstances like super optimal nitrogen supply or cutting, lodging, secondary panicles will additionally emerge from the base node of the tiller. A tiller has only one primary panicle but it can have

more than one secondary panicle. In the experiments two types of panicles were observed: primary and secondary panicles (Figure 16); where most of the secondary panicles appeared three weeks after the emergence of the primary panicles. In comparison to the primary panicles the contribution of the secondary panicles to the overall yield of a rice plant is minor due to immature seeds at harvesting time.

Figure 16. The primary and secondary panicles of the treated plant IR28 under non salt-stress conditions

The number of primary panicles per plant

EBl treatment increased the number of primary panicles of both varieties under 50 mmol NaCl conditions, but this increase was not significantly different. Under the 100 mmol salinity level and under the control conditions, the application of EBl did not affect the primary panicles in either variety (Table 24).

Secondary panicles

EBl application enhanced the number of secondary panicles in both varieties. The higher level of salt was the less number of secondary panicles (Table 23). In 50 mmol NaCl concentration, the number of secondary panicles of the salt-sensitive

variety IR28 was not different between EBI treated plants and untreated plants. However, treatment with EBI significantly increased the number of secondary panicles of variety MTL119 under non salt-stress condition. With the application of the 100 mmol NaCl concentration, the EBI treatment tended to increase the number of secondary panicles of both varieties. In addition, the secondary panicles of the treated plants appeared earlier than the untreated plants around 1 week; therefore, most of them were headed at harvest time while secondary panicles of the untreated plants were not headed. Thus, EBI application not only increases the number of secondary panicles but also induces their early appearance.

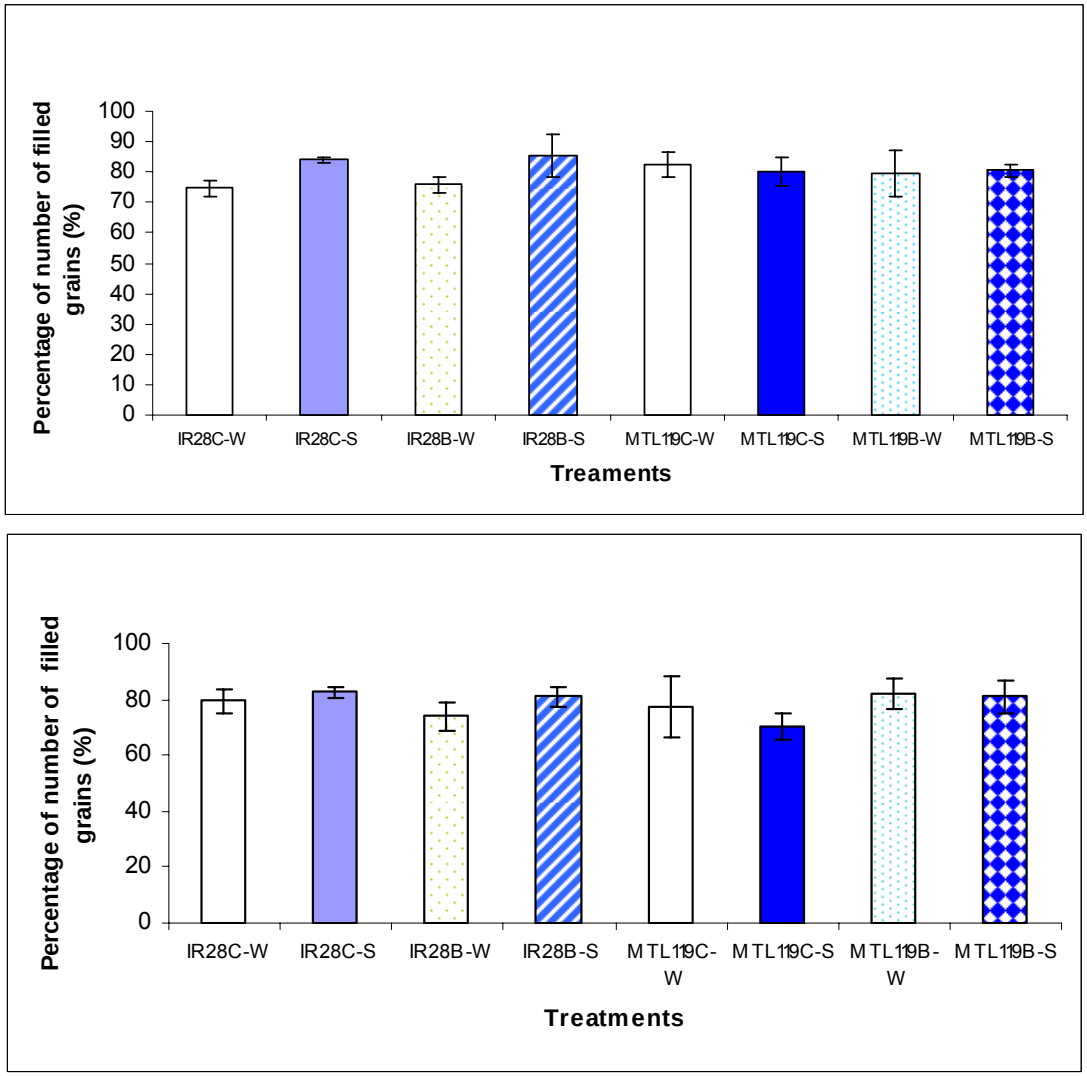
Table 23. Influence of 24-epibrassinolide application on number of secondary panicles of two varieties MTL119 and IR28 under control and salt-stress conditions

Variety	**Treatment**	**Secondary panicles per plant**	
		50 mmol NaCl	100 mmol NaCl
IR28	Control + water	7.78 ^{bc}	7.44 ^{a}
IR28	Control + salt	2.22 ^{efg}	0.22 ^{d}
IR28	EBI + water	9.11 ^{ab}	8.33 ^{a}
IR28	EBI + salt	2.33 ^{ef}	1.89 ^{cd}
MTL119	Control + water	3.00 ^{de}	2.78 ^{bc}
MTL119	Control + salt	0.00 ^{g}	0.00 ^{d}
MTL119	EBI + water	6.11 ^{c}	3.67 ^{b}
MTL119	EBI + salt	0.33 ^{fg}	0.22 ^{d}
P		< 0.05	< 0.05

Means in each column with the same letter are not significantly different at P<0.05

Number of grains per panicle

The number of grains per panicle of two cultivars under both conditions was not affected by EBI treatment, it was only reduced by salt-stress. Particularly, the number of grains per panicle of the salt-sensitive variety IR28 was more decreased under the higher salt-stress (Table 24).



C: control (without EBI); B : with EBI
S: salt-stress condition; W: water (non salt-stress condition)

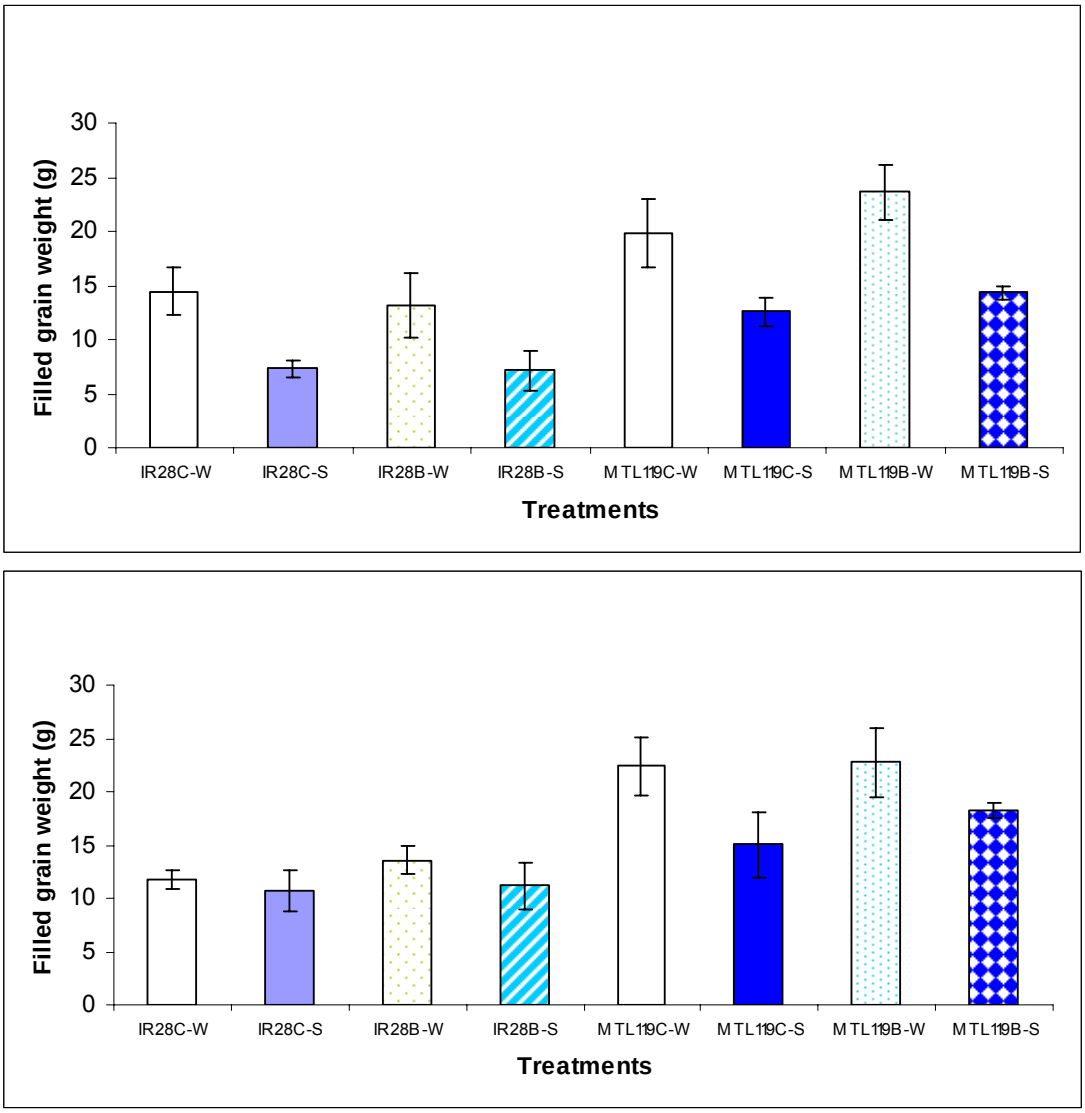
Figure 17. Influence of 24-epibrassinolide application on percentage of the number of filled grains per panicle of two varieties MTL119 and IR28 under control and with 50 mmol NaCl (upper graph) and 100 mmol (lower graph) conditions

Percentage of the number of filled grains per panicle

EBl application had no significant effect on the percentage of the number of filled grains per panicle in two varieties under both salt-stress and non salt-stress conditions. Particularly, under non salt-stress the percentage of the number of filled grains of the treated plants IR28 was lower than under salt-stress conditions (Figure 17). The number of filled grains per panicle was not significantly different between the EBI treated and untreated plants under both salt-stress and non-stressed conditions. The number of filled grains per panicle was only decreased by salt-stress, and the higher salt level was the stronger decrease (Table 24).

Grain weight, yield and harvest index

An EBl application had no significant effect on either the grain weight (Table 24) or the yield (Figure 18) of two varieties under both conditions when salt-stress was applied during tillering. The grain weight was only strongly affected by salt-stress; the higher salt concentration was the smaller size of grain. The application of EBl had no significant effect on the percentage of grain per total biomass weight of both varieties under non salt-stress and 50 mmol NaCl conditions. However, it significantly affected the harvest index of both genotypes under 100 mmol NaCl (Figure 19). In summary, when salt-stress is applied during the vegetative stage, EBl application significantly increases the proline accumulation of the salt-sensitive variety IR28, enhances the chlorophyll content of young leaves and reduces the ratio of injured-leaves of both varieties. However, it has no significant effect on the yield and yield components of both cultivars, even it increases the harvest index in 100 mmol salt concentration.



C: control (without EBl); B : with EBl; S: salt-stress condition; W: water (non salt-stress condition)

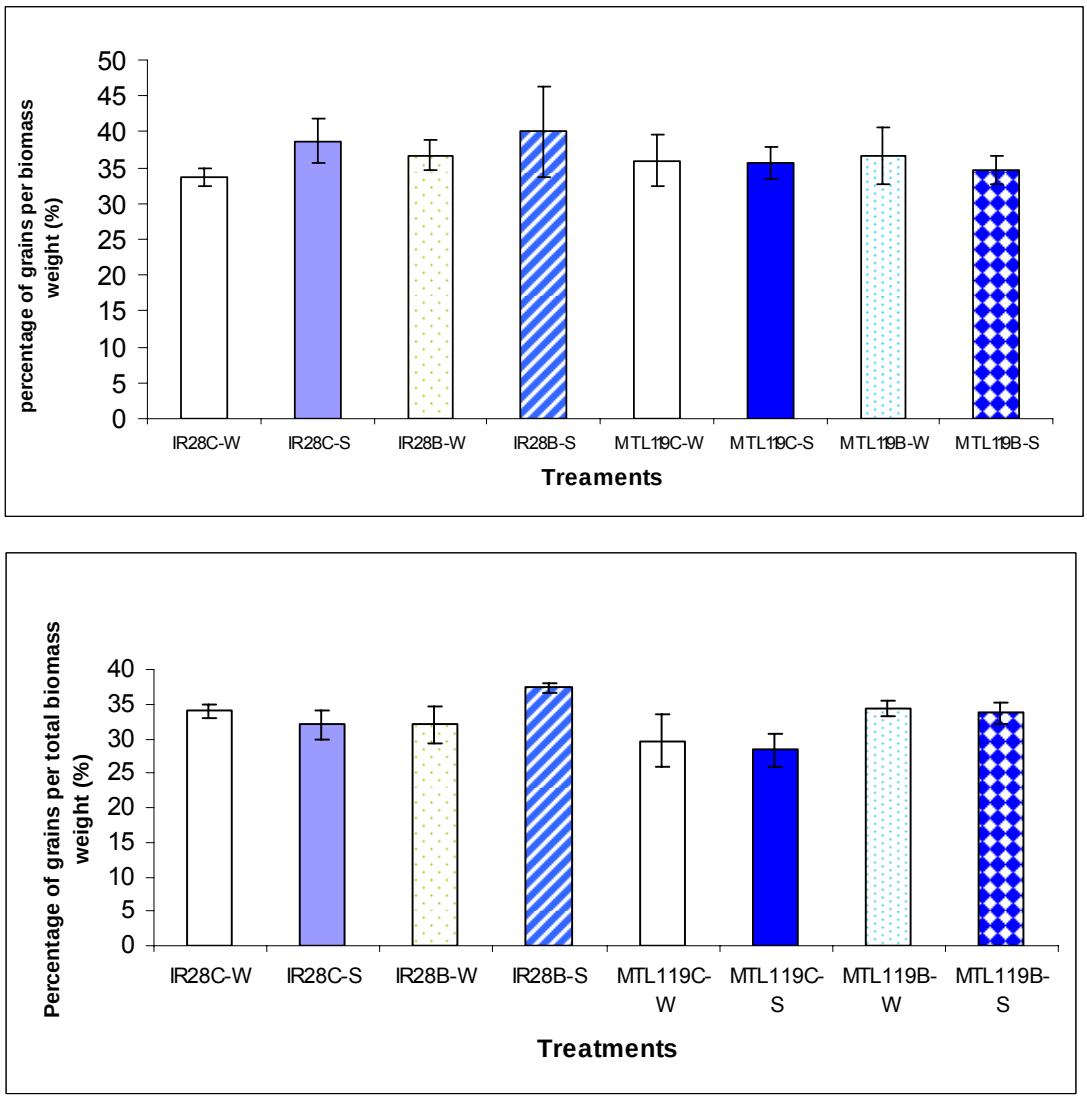
Figure 18. Influence of 24-epibrassinolide application on weight of filled grains per plant (yield) of varieties MTL119 and IR28 under control conditions and with 50 mmol NaCl (upper graph) and 100 mmol NaCl (lower graph)

Table 24. Influence of 24-epibrassinolide application on yield components of two varieties MTL119 and IR28 under controlled conditions and with 50 mmol NaCl (upper table) and 100 mmol NaCl (lower table)

Variety	**Treatment**	**Yield component**				
	50 mmol NaCl	Primary Panicles per plant	Number of grains per panicle	Percentage of filled grains per panicle (%)	Number of filled grain per panicle	Weight of 1000 grains (g)
IR28	Control + water	7.22	80.99	74.67	60.59	26.89
IR28	Control + salt	8.22	62.98	84.00	52.84	24.73
IR28	EBl + water	6.89	95.64	76.00	72.22	27.36
IR28	EBl + salt	8.56	61.71	85.33	52.59	24.79
MTL119	Control + water	7.55	111.43	82.33	91.79	32.26
MTL119	Control + salt	7.33	88.86	80.33	71.91	28.47
MTL119	EBl + water	7.67	116.30	79.33	92.48	32.03
MTL119	EBl + salt	9.00	88.37	80.67	71.44	28.44
P		ns	ns	ns	ns	ns

Variety	**Treatment**	**Yield component**				
	100 mmol NaCl	Primary Panicles per plant	Number of grains per panicle	Percentage of filled grains per panicle (%)	Number of filled grain per panicle	Weight of 1000 grains (g)
IR28	Control + water	6.78	100.05	79.33	79.20	26.92
IR28	Control + salt	6.89	55.81	82.67	46.03	23.16
IR28	EBl + water	6.56	100.81	74.00	74.38	26.87
IR28	EBl + salt	6.89	55.23	81.00	44.97	22.88
MTL119	Control + water	7.66	106.87	77.33	82.95	31.20
MTL119	Control + salt	7.56	91.71	70.33	64.09	25.99
MTL119	EBl + water	8.22	108.43	82.33	89.30	32.22
MTL119	EBl + salt	8.45	82.87	81.00	66.61	25.52
P		ns	ns	ns	ns	ns

ns: non significant



C: control (without EBl); B : with EBl
S: salt-stress condition; W: water (non salt-stress condition)

Figure 19. Influence of 24-epibrassinolide application on percentage of grains per total biomass weight of two varieties MTL119 and IR28 under non salt-stress conditions and with 50 mmol NaCl (upper graph) and 100 mmol NaCl (lower graph)

3.4. Effects of 24-epibrassinolide application on salinity tolerance of rice during the heading stage (experiments in potted soil)

3.4.1. Morphological characteristics

Results in Table 25 indicate that EBl treatment had no significant effect on the morphological characteristics including stem height, number of tillers, number of leaves and LAI of two rice varieties under both salt-stress and non-stressed conditions.

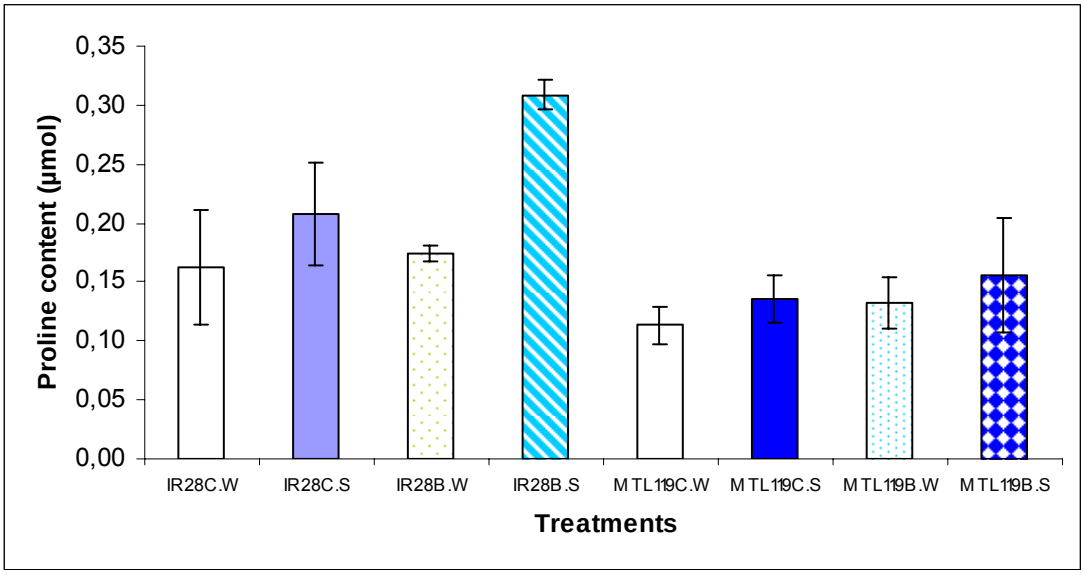
3.4.2. Physiological traits

Phenology

The flowering and harvesting date were not influenced by EBl treatment. The flowering and harvesting date of IR28 were 80 and 120 days after sowing, respectively; and for MTL119 were 120 and 155 days after sowing, respectively.

Proline

Results in Table 26 and Figure 20 show that treatment with the application of EBl significantly increased proline content of salt sensitive variety IR28 at the 10^{th} day after stress (Table 26). The proline concentration of EBI-treated plants was 0.31 µmol g^{-1} fresh leaves, compared to 0.21 µmol g^{-1} fresh leaves in untreated plants under 100 mmol NaCl concentration, an increase up to 48%. However, under non-stressed condition, proline accumulation of IR28 was not affected by EBl application. With the salt-tolerant variety MTL119, EBl treatment had no significant effect on proline content under both salt-stress and non-stress conditions. The proline content of the treated plants was 0.16 (µmol g^{-1} fresh leaves) in compared to 0.14 (µmol g^{-1} fresh leaves) of the untreated plants under 100 mmol NaCl concentration.



C: control (without EBl); B : with EBl; S: salt-stress condition; W: water (non salt-stress condition)

Figure 20. Influence of 24-epibrassinolide application on proline accumulation of two varieties MTL119 and IR28 at the heading stage under non salt-stress and 100 mmol NaCl conditions

Table 25. Influence of 24-epibrassinolide application on morphological characteristics of two varieties MTL119 and IR28 at the heading stage under control conditions and with 50 mmol NaCl (upper table) and 100 mmol NaCl (lower table)

Variety	**Treatment**	**Morphological characteristics**			
	50 mmol NaCl	Stem height (cm)	Number of tillers	Number of leaves	LAI
IR28	Control + water	100	9.22	27.67	2.77
IR28	Control + salt	99	9.00	22.89	2.45
IR28	EBI + water	100	9.78	29.00	2.71
IR28	EBI + salt	97	10.11	26.67	2.66
MTL119	Control + water	99	9.67	32.11	2.91
MTL119	Control + salt	96	8.44	31.22	2.71
MTL119	EBI + water	97	9.56	29.78	2.69
MTL119	EBI + salt	100	9.78	33.11	2.93
P		ns	ns	ns	ns

Variety	**Treatment**	**Morphological characteristics**			
	100 mmol NaCl	Stem height (cm)	Number of tillers	Number of leaves	LAI
IR28	Control + water	102	10.00	26.78	2.80
IR28	Control + salt	97	9.44	22.56	2.02
IR28	EBI + water	101	9.22	25.67	2.61
IR28	EBI + salt	97	9.33	23.56	2.18
MTL119	Control + water	98	10.11	32.89	2.91
MTL119	Control + salt	98	9.77	35.23	3.08
MTL119	EBI + water	96	9.55	29.56	2.54
MTL119	EBI + salt	98	10.00	29.78	2.66
P		ns	ns	ns	ns

ns: non significant

Table 26. Influence of 24-epibrassinolide application on proline content of third leaves of two varieties MTL119 and IR28 at the heading stage under non salt-stress and 100 mmol NaCl conditions

Variety	Treatment	**Proline** (µmol g^{-1} fresh leaves)	
		1 DBS-S	**10 DAS-S**
IR28	Control + water	0.178	0.163 ^{cde}
IR28	Control + salt	0.152	0.207 ^{bc}
IR28	EBI + water	0.185	0.174 ^{cd}
IR28	EBI + salt	0.215	0.308 ^{a}
MTL119	Control + water	0.173	0.113 ^{e}
MTL119	Control + salt	0.128	0.136 ^{de}
MTL119	EBI + water	0.148	0.133 ^{de}
MTL119	EBI + salt	0.153	0.156 ^{de}
P		ns	< 0.01

Means in each column with the same letter are not significantly different at P<0.01
DBS-S: day before salt-stress (100mmol), DAS-S: day after salt-stress
ns: non significant

3.4.3. Yield and yield components

Number of primary panicles

The number of primary panicles per plant was not affected by EBI under 50 mmol NaCl concentration and non-stress conditions. However, in 100 mmol NaCl concentration, EBI treatments significantly decreased the number of primary panicles per plant of variety MTL119 (Table 27 and Table 28).

Number of secondary panicles

The EBI treatment increased the number of secondary panicles of the salt-sensitive genotype IR28 under both cases with or without salt. However, the number of secondary panicles of the salt-tolerant variety MTL119 was not affected by EBI application under both conditions (Table 29). On the other hand, the secondary panicles of the EBI treated plants under non-stress conditions appeared one week earlier than those of the untreated plants.

Number of grains per panicle and the percentage of number of filled grains per panicle

Results of Table 27, Table 28 and Figure 21 indicate that EBl treatment had no significant effect on number of grains per panicle and the percentage of number of filled grains per total grains of both varieties under non salt-stress and salt-stress conditions. However, this parameter was increased in variety MTL119 under 50 mmol salt concentration. The percentage of number of filled grains per total grains of IR28 under normal conditions was lower than under salt-stress conditions.

Number of filled grains per panicle

EBl application had no effect on the number of filled grains per panicle of variety IR28 under both non-stressed and salt-stress conditions. However, EBl treatment increased the number of filled grains per panicle of the treated MTL119 plants under salt-stress conditions, an increase up to 18-20%, as shown in Table 28 and Figure 22. The EBl treatment induces the number of filled grains per panicle of MTL119 when salt-stress is applied at heading period.

Table 27. Influence of 24-epibrassinolide application on yield components of two varieties MTL119 and IR28 under non salt-stress and 50 mmol NaCl during heading stage

Variety	Treatment	Yield component				
		Primary Panicles per plant	Number of grains per panicle	Percentage of filled grains per panicle (%)	Number of filled grain per panicle	Weight of 1000 grains (g)
IR28	Control + water	9.11	78.52	71.33	56.00	27.37
IR28	Control + salt	8.89	73.57	78.00	57.67	26.89
IR28	EBl + water	9.11	80.13	70.67	56.67	27.79
IR28	EBl + salt	10.00	72.03	79.00	57.33	27.39
MTL119	Control + water	9.89	75.06	63.00	47.00	26.88
MTL119	Control + salt	9.67	72.48	55.00	40.00	26.29
MTL119	EBl + water	9.67	80.41	62.67	46.67	26.39
MTL119	EBl + salt	10.11	76.92	64.67	48.33	26.89
P		ns	ns	ns	ns	ns

ns: non significant

Table 28. Influence of 24-epibrassinolide application on yield components of two varieties MTL119 and IR28 under non salt-stress and 100 mmol NaCl during heading stage

Variety	Treatment	Yield component				
		Primary Panicles per plant	Number of grains per panicle	Percentage of filled grains per panicle (%)	Number of filled grain per panicle	Weight of 1000 grains (g)
IR28	Control + water	10.22 ^{c}	73.92	66.67	49.33	27.30
IR28	Control + salt	9.45 ^{ef}	72.27	80.67	58.33	26.69
IR28	EBl + water	9.11 ^{f}	75.34	67.67	51.00	27.39
IR28	EBl + salt	9.67 ^{de}	77.38	80.33	59.67	26.82
MTL119	Control + water	10.66 ^{b}	72.99	61.00	44.33	26.88
MTL119	Control + salt	11.00 ^{ab}	67.88	62.00	42.00	26.61
MTL119	EBl + water	9.78 ^{d}	70.61	61.67	43.33	26.78
MTL119	EBl + salt	10.22 ^{c}	76.00	65.67	49.33	26.62
P		< 0.05	ns	ns	ns	ns

Means in each column with the same letter are not significantly different at P<0.05
ns: non significant

Table 29. Influence of 24-epibrassinolide application on number of secondary panicles of two varieties MTL119 and IR28 under non salt-stress and salt-stress during heading stage

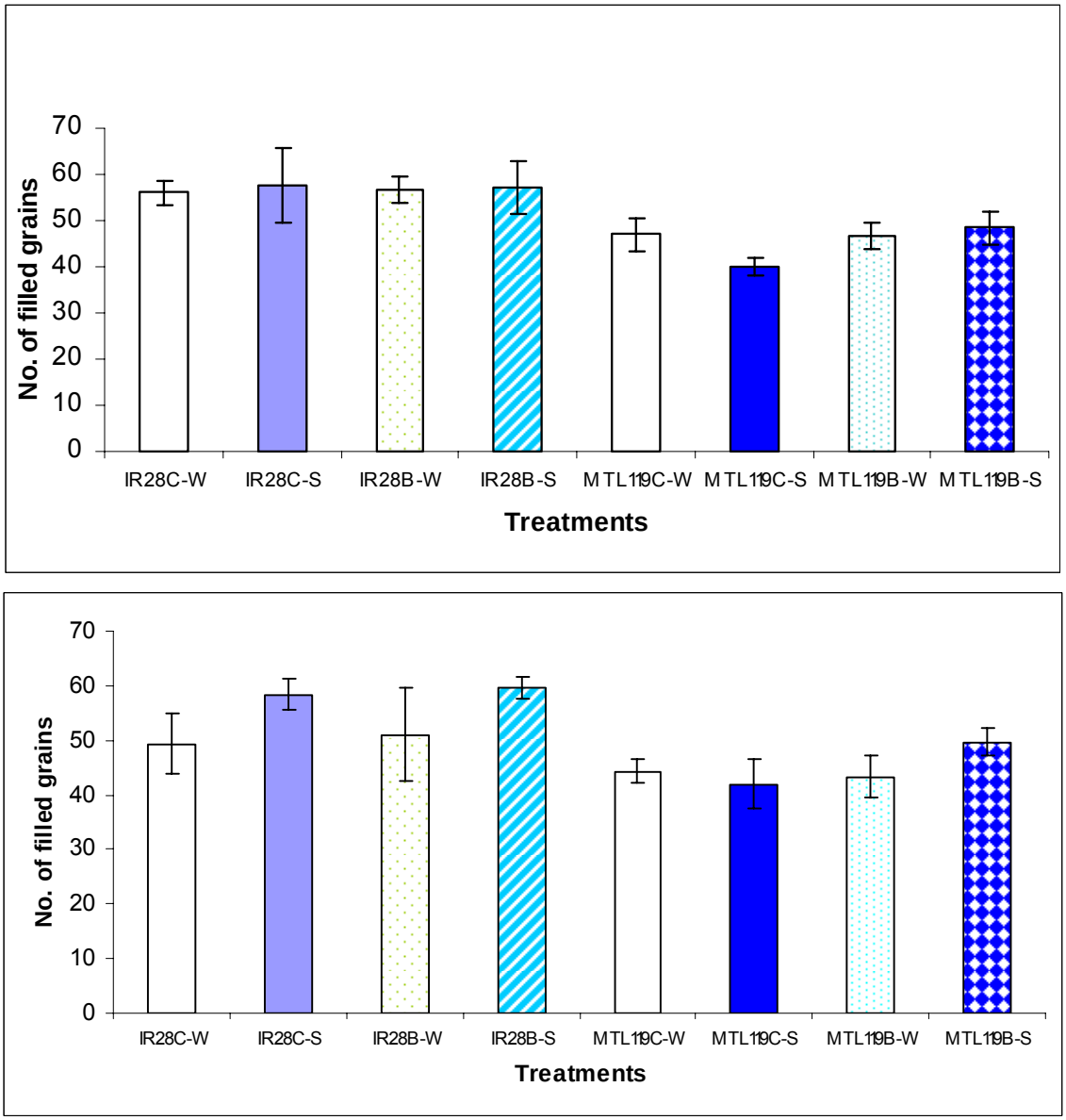
Variety	Treatment	Secondary panicles per plant	
		50 mmol NaCl	**100** mmol NaCl
IR28	Control + water	4.33 bc	3.33 b
IR28	Control + salt	1.67 c	0.22 c
IR28	EBl + water	8.00 a	8,67 a
IR28	EBl + salt	3.67 b	1.89 bc
MTL119	Control + water	9.33 a	9.78 a
MTL119	Control + salt	8.33 a	8.33 a
MTL119	EBl + water	9.22 a	8.45 a
MTL119	EBl + salt	9.11 a	8.67 a
P		< 0.05	< 0.05

Means in each column with the same letter are not significantly different at P<0.05

Grain weight and yield

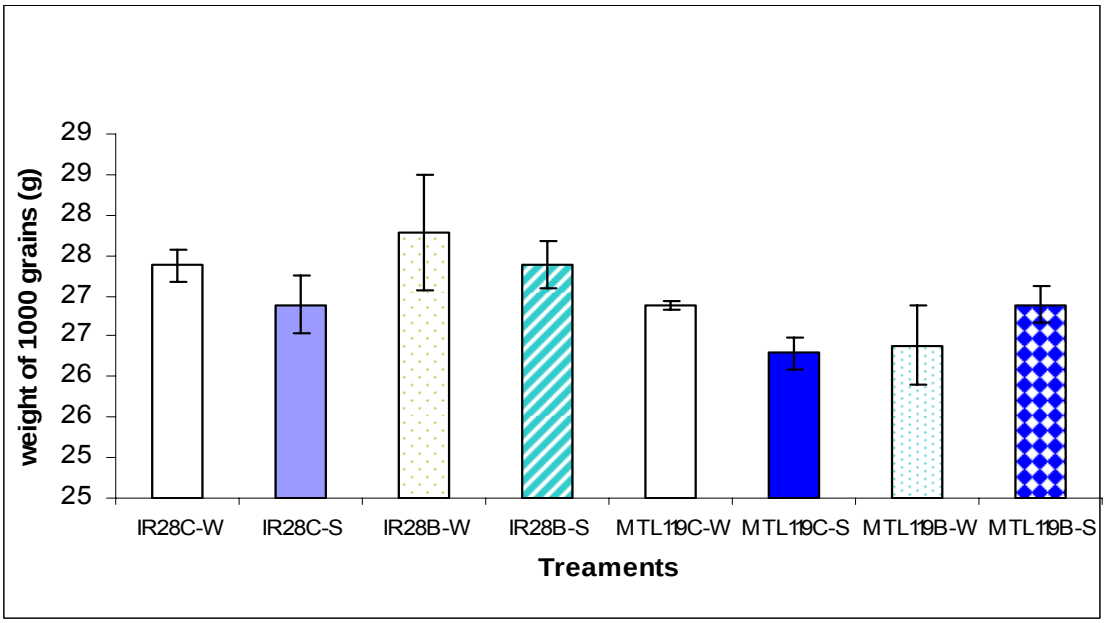
EBI treatment increased the grain weight of the treated plants of both varieties under the lower salt-stress level (50 mmol), the increase of the grain weight of IR28 and MTL119 was 1.9% and 2.3%, respectively. However, it was significantly different only the grain weight of the salt-tolerant variety MTL119 (2.3%). Particularly, under non-stressed condition EBI treatment had no significant effect on the grain size of both varieties (Figure 22). On the other hand, the grain size of the salt-sensitive genotype IR28 was significantly decreased by salt-stress. The higher the salt concentration was, the smaller was the grain size. However, the grain size of EBI treated plants was not significantly different between under non-stress and salt-stress conditions. In addition, the grain size of MTL119 is normally bigger than that of IR28 variety but due to low humidity the grain size of MTL119 was smaller than IR28 variety in this experiment. The EBI application significantly increases the grain size up to 2.3% of the salt-tolerant variety MTL119 in 50 mmol NaCl level.

The application of EBI increased the yield of both varieties under two salt-stress levels. However, the higher the salt concentration was the less effect of EBI treatment. In detail, EBI application increased the weight of filled grains per plants of the salt-sensitive IR28 up to 14% under 50 mmol NaCL and up to 5% under 100 mmol NaCl (Table 30 and Figure 23). The yield of the EBI treated plants of MTL119 increased up to 30% under 50 mmol NaCL and 9% under 100mmol NaCl, respectively. However, the yield of EBI treated plants of both varieties under non salt-stress conditions was not higher than that of untreated plants. Consequently, EBI application reduced the damage of salt-stress on rice and increased the grain yield. Its effects are significantly different from light salt-stress conditions, which are around 50 mmol NaCl concentration, or starting of stress at the late growth stadium after the heading stage.



C: control (without EBl); B : with EBl; S: salt-stress condition; W: water (non salt-stress condition)

Figure 21. Influence of 24-epibrassinolide application on Number of filled grains per panicle of two varieties MTL119 and IR28 under control and with 50 mmol NaCl (upper graph) and 100 mmol NaCl (lower graph) during heading stage



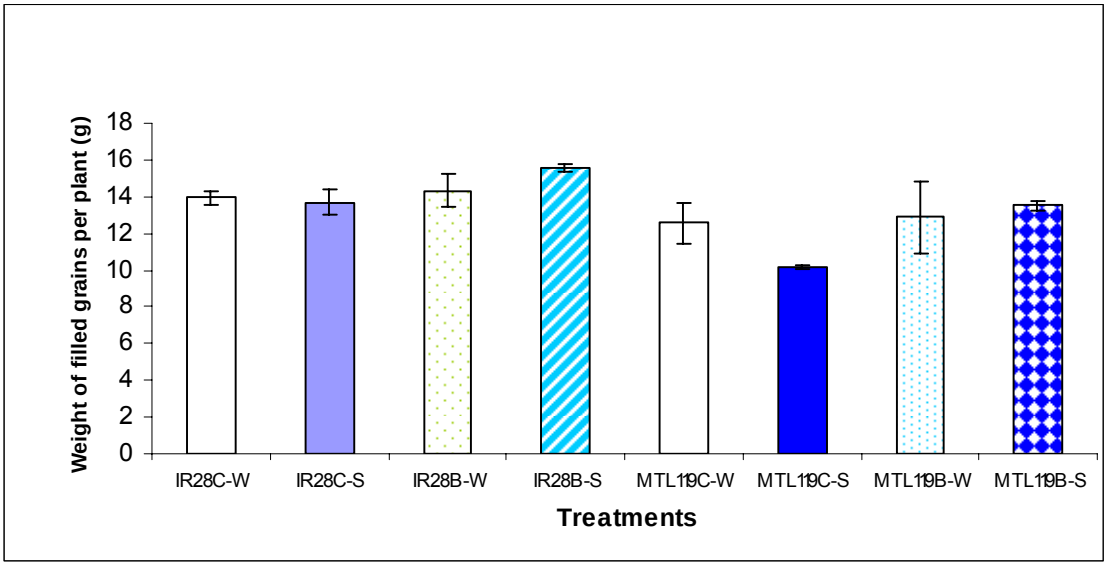
C: control (without EBl); B : with EBl; S: salt-stress condition; W: water (non salt-stress condition)

Figure 22. Influence of 24-epibrassinolide application on grain weight of two varieties MTL119 and IR28 under non salt-stress and 50 mmol NaCl during heading stage

Table 30. Influence of 24-epibrassinolide application on yield of two varieties MTL119 and IR28 under non salt-stress and salt-stress during heading stage

Variety	**Treatment**	**Yield** (g plant^{-1})	
		50 mmol NaCl	100 mmol NaCl
IR28	Control + water	13.93 ^{bc}	13.80
IR28	Control + salt	13.68 ^{bc}	14.71
IR28	EBl + water	14.32 ^{b}	12.73
IR28	EBl + salt	15.55 ^{a}	15.48
MTL119	Control + water	12.59 ^{de}	12.75
MTL119	Control + salt	10.11 ^{f}	12.30
MTL119	EBl + water	11.94 ^{e}	11.38
MTL119	EBl + salt	13.10 ^{cd}	13.55
P		<0.05	ns

Means in each column with the same letter are not significantly different at P<0.05



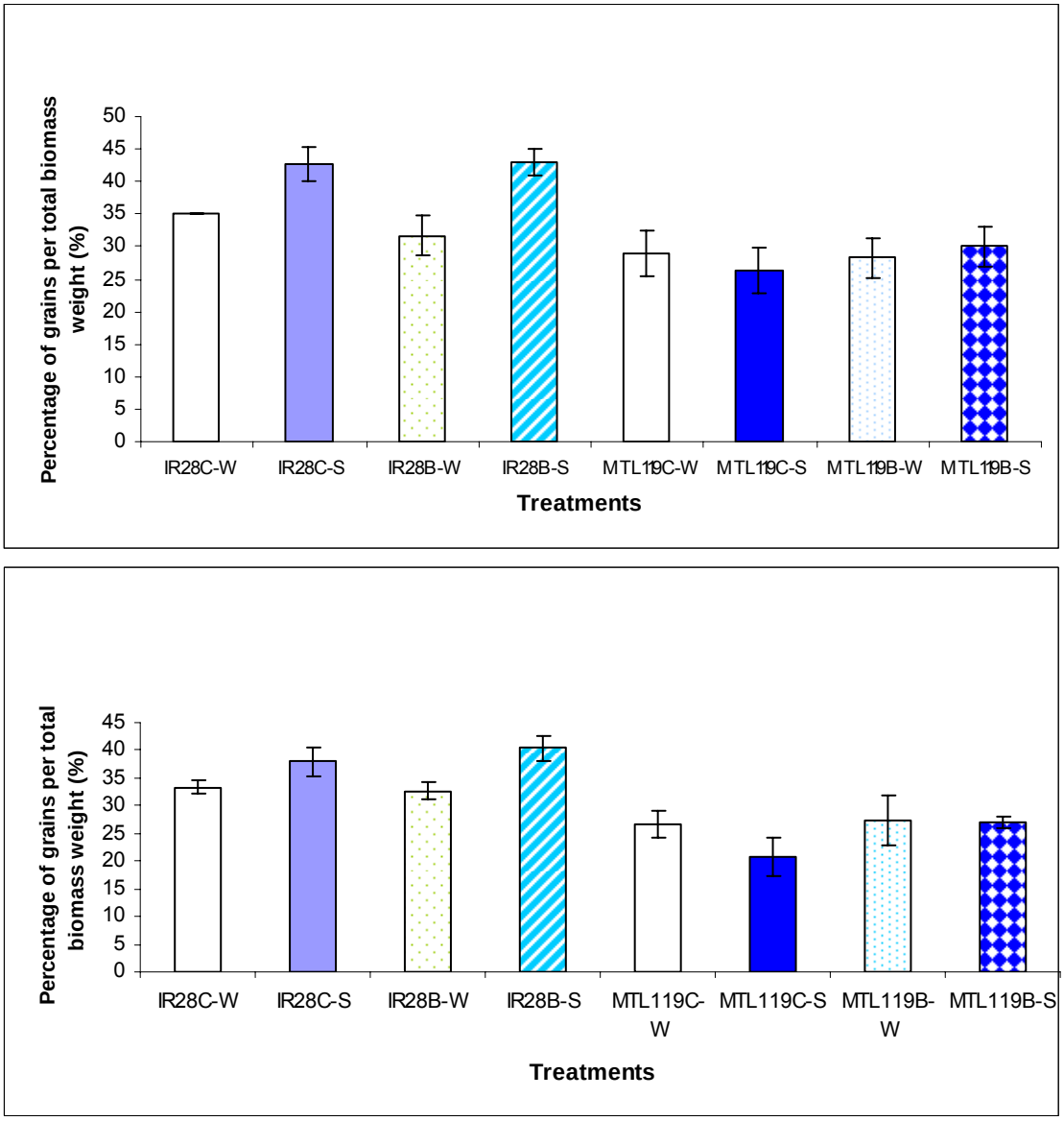
C: control (without EBl); B : with EBl; S: salt-stress condition; W: water (non salt-stress condition)

Figure 23. Influence of 24-epibrassinolide application on weight of filled grains per plant of two varieties MTL119 and IR28 under non salt-stress and 50 mmol NaCl during heading stage

The ratio of grains per total biomass weight (Harvest index)

Under normal conditions, EBI treatment had no significant effect on the ratio of grains per total biomass weight of both varieties, but under 50 mmol salt level EBI application increased this trait of both genotypes. Particularly, it significantly increased (7%) harvest index of variety MTL119 variety under salt-stress conditions (Figure 24).

Presented data show that EBI application has some effect on salinity-affected rice plants during the reproductive stage. Thus, EBI can increase the yield of the salt tolerant variety MTL119 by 30% and that of the salt sensitive variety IR28 by 14%. These yield increases are associated an increase in the number of filled grains per panicle (20%), the grain size (2.3%) and harvest index (7%) of the salt tolerant variety MTL119 with 50 mmol NaCl level. The mechanism associated with this increased salinity tolerance appears to be related to the induction of increased proline concentration in leaf tissues.



C: control (without EBl); B : with EBl; S: salt-stress condition; W: water (non salt-stress condition)

Figure 24. Influence of 24-epibrassinolide application on the ratio of grains per total biomass weight of two varieties MTL119 and IR28 under control and with 50 mmol NaCl (upper graph) and 100 mmol NaCl (lower graph) during heading stage

4. DISCUSSION

4.1. Effects of 24-epibrassinolide application on salinity tolerance during germination and seeding stages

Naturally, a high seed germination rate will lead to a higher ratio of healthy seedlings, the lowering of total amounts of seeds needed for sowing, and consequently having an influence on the yield of crops (Kamuro, 1999). Therefore, the promotion of seed germination is an important factor in rice cultivation. The result of the accomplished experiments (see Section 3.1) confirmed the promoting effect of EBl on the germination of rice seeds. EBl increased the seed germination rate of fresh seeds of the salt-tolerant variety MTL119 up to 25% under non-salt-stress and up to 50% under salt-stress conditions. However, EBl had no effect on aged seeds of MTL 119 (older than 3 weeks) regardless their age there was no effect on seeds of the salt-sensitive variety IR28.

By considering the mechanism of germination of monocot seeds as explained by Koning (1994), this finding may be elucidated. Germination is initiated by water and reasonably warm temperature. The water passes through the embryo, picks up the germination signal: the hormone gibberellic acid (GA) and moves the hormone from the embryo to the aleurone layer of the endosperm. This layer of cells stores high protein. The water activates hydrolysis enzymes that degrade the storage protein into amino acids. The gibberellic acid activates the DNA gene coding for the enzyme amylase in the aleurone cells result in producing amylase inside the aleurone cells. Then amylase is secreted from the aleurone layer into the endosperm to catalyze the hydrating reaction of stored starch into sugar. The sugar happens to be glucose, which is transported to the embryo. The sugar fuels respiration in the embryo so it can grow (Koning, 1994). Seed dormancy and germination are regulated by the plant hormones abscisic acid (ABA) and gibberellic acid (GA). ABA induces seed dormancy in maturing embryos and inhibits germination of seeds while GA breaks seed dormancy and promotes germination, and EBl has a role in overcoming inhibition of germination by ABA (Beaudoin *et al.*, 2000; Debeaujon and Koornneef, 2000; Steber and Mccourt,

2001). So, pre-treatment of seeds with EBI enhances the total germination rate because EBI counteracts the effects of ABA, stimulating the activity of GA. Seed dormancy highly depends on genotypes and storage times after harvesting, e.g. seeds of IR28 possess no dormancy at all and gain high germination rates already a few days after harvesting; conversely seeds of MTL119 fall into an at least 3 weeks long dormancy period after maturity. In the accomplished experiments, the germination rate of IR28 and aged MTL119 seeds was not significantly affected by EBI. These non-effects are probably due to the absence of seed dormancy within these seeds which constitutes the main target of EBI for increasing the germination rate. Since these seeds already germinated at maximum rate EBI could not increase this rate additionally. Only in the fresh MTL 119 seeds where still a persisted high dormancy level was present, EBI increased the germination rate by lowering seed dormancy.

However, Yamaguchi *et al.* (1987) found that brassinolide improves also the germination and seedling emergence of - aged rice seeds. Perhaps, the difference between the results here shown and Yamaguchi's findings are due to following reasons: In this work seeds were called aged already 3 weeks after harvesting and the oldest ones have been aged for 3 months. In Yamaguchi studies presumably older seeds were used. Another reason, probably, was that they have been stored here for 3 months under mostly ideal conditions, resulting in a good chance for stimulating germination rate at a very high level.

Normally, the germination rate of rice seeds will decrease continuously with prolonged times of storage and this process depends not only on the time but also on the conditions, e.g. in normal tropical conditions germination rate of rice decreases after 6 months (Le, 1999). The present study indicated that a positive effect of EBI treatment on seed germination only occurred in very fresh seeds of MTL119 (high dormancy) but not in stored seeds with low germination inhibition. This suggests that EBI can promote the germination of dormant rice seeds.

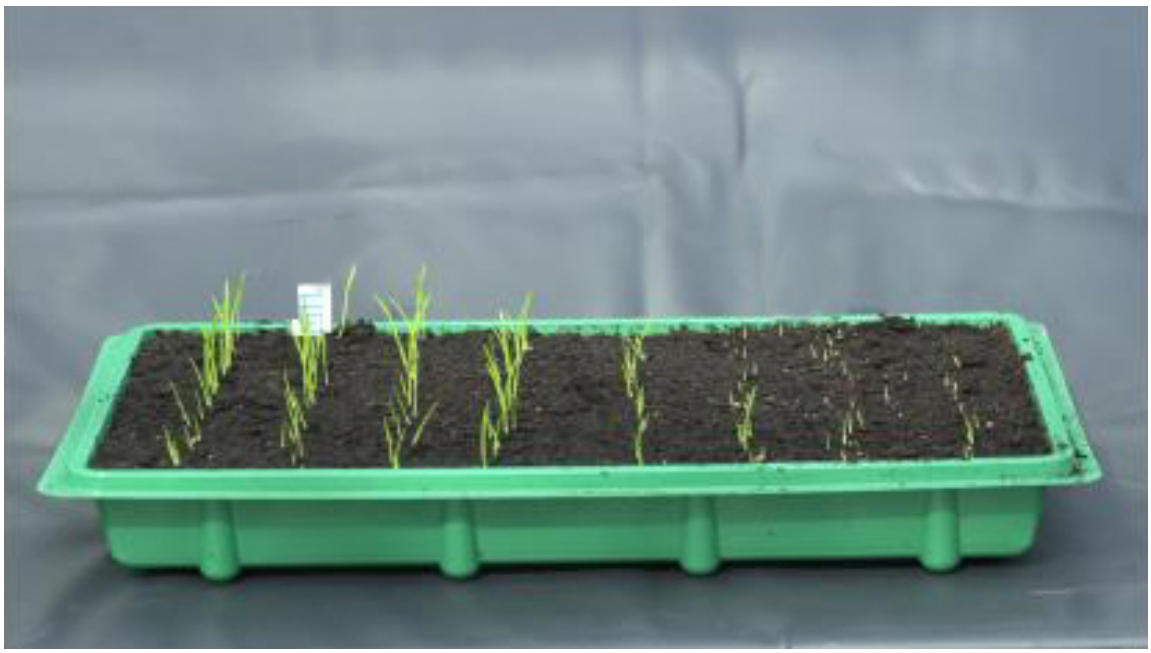


Figure 25. Effect of EBI on germination and seedling growth of variety MTL119 under non salt-stress. The four rows on the left are EBI treated seeds. The four rows on the right are non-EBI treated seeds.

Besides, salt-tolerant varieties, such as the MTL119, are capable of tolerating salt stress at vegetative and reproductive stage and are only sensitive at the germination stage in comparison to salt sensitive IR28. MTL119 seeds are hypothesized to contain higher ABA concentration resulting in a higher dormancy, coupled with a lower germination rate of MTL119 in contrast to IR28 seeds under salt-stress conditions. According to Xiong *et al.*, (2002), ABA-deficient rice is more tolerant to salt stress at germination stage but is hypersensitive to salt stress at vegetative stage, as shown for IR28 variety. Therefore, this may be a reason why MTL119 is more sensitive to salinity than IR28 at germination stage. IR28 seeds as the non dormant variety may have lower ABA content, and MTL119 seeds, as the dormant type, have higher ABA contents. However, EBI enhanced germination rate of the fresh seeds of variety MTL119 under salt-stress as well as under normal control conditions, whereas it had no effect on IR28. Similar results were reported by Anuradha and Rao (2001), Rao *et al.* (2002), and Anuradha and Rao (2003) where treatment of rice seeds with EBI and 28-homobrassinolide increased the

germination rate under salt-stress conditions. Thus, EBI has an important role in promoting germination of moderately dormant seeds under saline as well as under normal conditions.

A normally germinated rice seed has two different types of roots: a primary root and secondary roots. The single primary root is initiated 2 to 3 days after seed imbibition, and the multiple secondary roots are formed 3 to 5 days after imbibition. The curving and shortening of the primary root of EBI treated seeds (see Section 3.1) clearly indicates the inhibitory effects of EBI on the growth elongation of the primary root axis. The same results were obtained with several concentrations of EBI from 0.01 to 10 mg l^{-1}. This inhibition phenomenon may be explained by the role of ethylene in root development. Typically, root growth is stimulated by low concentration of ethylene (< pmol), and inhibited at higher concentration (Reid and Ross, 1995). Arteca (1995) particularly confirmed the inhibitory effects on expansion of high ethylene concentration. The BR-induced symptoms could be coupled with the higher production of ethylene in stem tissue (Wang *et al.*, 1993). Despite of inhibiting the elongation of the primary root, EBI was found to promote the formation of secondary roots (Fig. 10). The secondary roots of treated seeds appeared 3 days earlier than those of the untreated seeds. This is probably one of the reasons why EBI is able to induce seedling growth and increases the ratio of healthy seedlings (Table 9). The same effects were observed by Chen (1990), while incubating tobacco explants on MS medium containing 0.01 - 0.05 mg l^{-1} EBI, the number of rootlets increased significantly (Zhao and Chen, 2003).

4.2. Effects of 24-epibrassinolide application on salinity tolerance during vegetative and reproductive stages

The morphological traits of the two employed varieties such as plant height, number of tillers and the LAI (leaf area index) were not significantly affected by EBl neither at the development stages of seedling nor at the heading stage. The following factors may explain these symptoms: the density of rice plants in this experiment was lower 23.5 plants m^{-2} compared to 33 plants m^{-2} in the normal field conditions meaning they develop different than under high plant density, and the illumination conditions in the greenhouse were not equally distributed over the tables (Fig. 6) as well as there was a strong influence of changing sunlight, and mobile lamp system, so that possible effects of EBI on the morphological traits got hidden under the stronger influence of the instable environmental effects.

In the study on EBI effects on photosynthesis, EBI reduced the damage of salt on leaf chlorophyll, but showed no effect on photosynthesis parameters such as CO_2 assimilation rate (Pn), Electron Transport (ET) and Quantum Efficiency (QE) (session 3.3.2). Perhaps, the results of these measurements were strongly affected by the presence of the mobile lamps employed in the greenhouse, which is presented in Fig. 6, as well as from the sometimes quickly changing sunlight resulting in greatly varied levels of photosynthetic active radiation (PAR) in the greenhouse. Yu *et al.,* (2004) demonstrated that EBl significantly increased the light saturated net CO_2 assimilation rate from 3-7 h after spraying with 0.1 mg l^{-1} EBl in *Cucumis sativus*. Increased CO_2 assimilation rate in EBl treated leaves was accompanied by the increase in the maximum carboxylation rate of Rubisco, and a higher quantum yield of PSII electron transport, mainly due to a significant increase in the photochemical quenching and unchanged efficiency of energy capture by open PSII reaction centers. EBl did not influence photorespiration (Yu *et al.*, 2004); however, from our experiments, there is no evidence to support that EBl has similar effects on the photosynthesis of rice. Therefore, further researches are recommended to confirm the role of EBl in photosynthesis efficient of rice.

In the study on leaf chlorophyll content, the application of EBI permitted to recover the chlorophyll loss of the youngest leaves of 50 days old rice plants under salt-stress conditions at the time of 3 weeks after salt stress application. It is noted that EBI did not affect the old leaves. Because the salt stress firstly damages the top leaves as being the youngest leaf. At the first impact of salt the tips of the top leaf discolor, then after one week under saline conditions the top leaves are rolled and completely discolored. From that point on the so-called secondary damage gradually spreads down to the old leaves. However, also after 3 weeks of saline conditions the old leaves were only slightly damaged, meaning they were still green and only the tips were wilted. However, the healthy old leaves of both varieties were always greener under salt stress situation than those of the plants in non-salt stress conditions. This was due to the fact that the chlorophyll content of the old leaves under salt stress conditions was higher than that under control. In these experiments no effect of EBI on the chlorophyll content could be observed.

The situation is different for the youngest leaves, which were severely injured and discolored at 3 weeks after salt stress. In this case, an EBI treatment recovered the youngest leaves from the damage received from the saline conditions; finally leading to higher chlorophyll content in the youngest leaves of EBI treated plants in comparison to non EBI treated plants.

The evaluation of morphological salt tolerance (see Session 3.3.2, Figure 12), showed not only that the damage caused by salt stress on the EBI treated plants was lower, but also that it occurred at a later point in time than in untreated plants. This means EBI reduces the damage of salt stress also by shifting the time frame in which first signs of salinity get visible on the leaves further and additionally by recovering the chlorophyll loss and stabilizing chlorophyll content in the leaves. As noted by Mohan (2000), the chlorophyll stability is an indication of the stress tolerance capacity of plants in general. In addition, these results follow the results of Wang (1997) who observed a significant increase of total chlorophyll content in rice leaves after brassinosteroid treatment and the observations of Anuradha and Rao (2003) that brassinosteroids remove the inhibitory effects of salt stress on pigment levels and that this could be one of the reasons for the noticed growth

stimulation induced by brassinosteroids under saline conditions. Furthermore, Sairam (1994) reported that water stress suffering wheat plants when treated with 28-homobrassinolide showed a higher chlorophyll level in their leaves, and Castle (2003) confirmed that EBI improves the tolerance of barley leaf to salt stress, reducing the damage by protecting cell ultrastructure and chloroplast membrane system (Castle *et al.*, 2003).

The accumulation of free proline as a widespread response to stresses is well-documented and not only common to plants, but also eubacteria, protozoa, marine invertebrates and algae make use of its beneficial properties (Skriver and Mundy, 1990). Proline is known as a compatible solute playing a role in the osmotic adjustment of the plant. It protects plants against the effects of water stress regardless if caused by drought or excess of salt or temperature extremes. Several studies found that proline accumulation is a response of plants to osmotic stress. Proline has the capability to protect membranes and proteins against the adverse effects of high concentrations of inorganic ions and temperature extremes (Pollard and Wynjones, 1979; Paleg *et al.*, 1981; Santoro *et al.*, 1992; Santarius, 1994). Proline is discussed to function as a membrane stabilized by replacing OH-group of water deficiency due to salt-stress conditions. Thus, proline is able to build up hydrogen bridges in order to protect sensitive cell structures. For this reason many plants accumulate proline as a non-toxic protective osmolyte (Binzel *et al.*, 1987; Mattioni *et al.*, 1997). The accumulation of proline in response to salinity stress is primarily localized in the cytosol of the plants (Pahlich *et al.*, 1983).

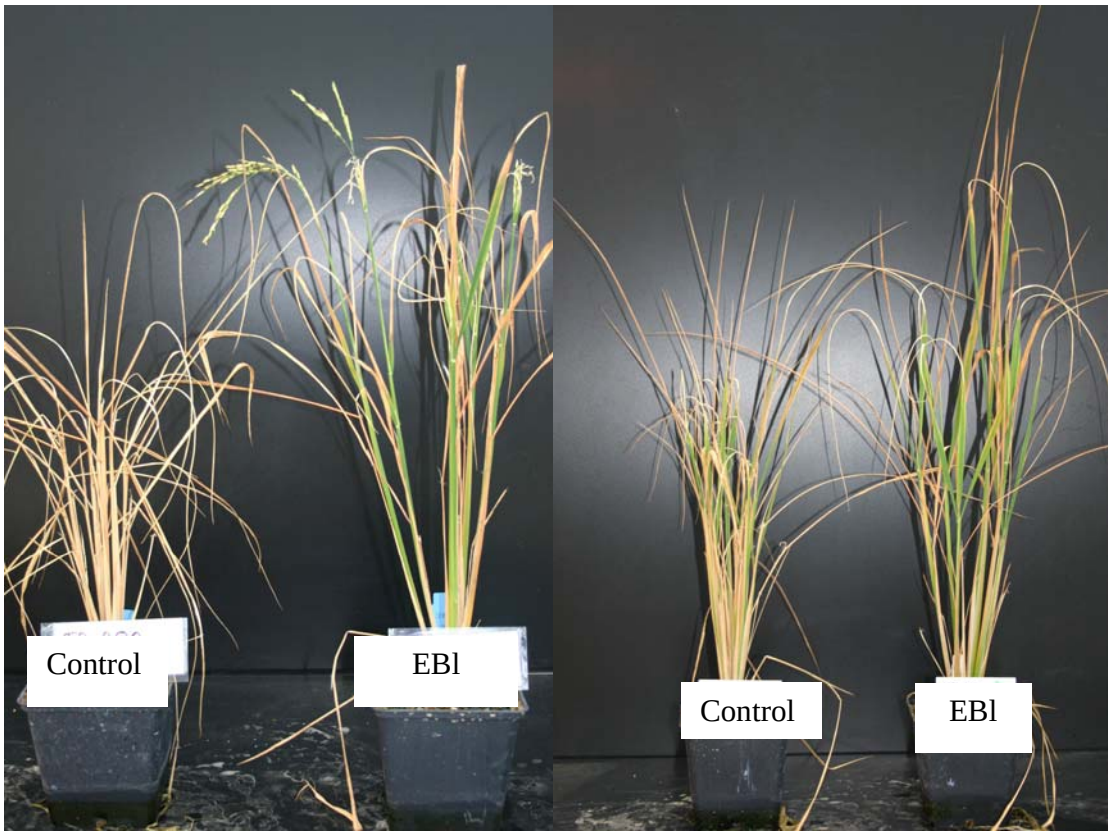
The experiments documented in this work confirmed an increase of proline accumulation in both rice cultivars under saline conditions. In fact, it was found that proline accumulation occurred in higher ratios in the salt-sensitive cultivar IR28 in contrast to the salt-tolerant cultivar MTL119. Under EBI treatment the proline content increased even more significantly in the cultivar IR28 under salt-stress conditions. This result could be obtained in different developmental stages like seedling stage, tillering and heading stage. However, the increase of proline content in the tillering stage was continued up to the 3rd week after salt-stress

application, then the content decreased back to nearly the same level as in the untreated plants (Fig. 14).

However, EBI did not significantly influence proline accumulation in the salt-tolerant variety MTL119 regardless if salt stressed or not. The fact that the increase of proline accumulation in the salt-sensitive variety IR28 was higher than that in the salt-tolerant variety MTL119 in saline conditions might be explained by the properties of IR28. As a salt-sensitive variety, it is probably not able to prevent sodium uptake into the roots as well as it cannot prevent the transfer of sodium into the shoot and leaves. Therefore, it is possible that high sodium content persists in leaves of IR28 under the pressure of saline environment, so that compatible solutes such as proline increased in leaves of IR28 in order to protect them against the damage of increasing salt concentrations. Conversely, MTL119 as the salt-tolerant variety may be able to control the salt uptake and transfer to the upper parts of plant avoiding, in this way, critical concentrations inside the leaves. That might be a reason why the accumulation of proline in the salt-tolerant variety MTL119 is lower than in the salt-sensitive IR28 variety.

The above findings support the observations of several researches on the effect of EBI on proline accumulation in plants. So, Liu and Zhu (1997) found that the salt-overly-sensitive *sos1 Arabidopsis* has an increased capability to accumulate proline in comparison to wild type plants. Interestingly, the enhanced resistance to salt-stress was attributed to BR-induced effects on membrane stability and osmoregulation (Wang *et al.*, 1993). In rice, 24-epibrassinolide treatment caused a reduction of oxidative stress markers, which normally coincides with abiotic stress. Oxidative stress markers like electrolyte leakage, malonedialdehyde content, and activity of superoxide dismutase decreased, but the levels of ATP and proline were enhanced (Clouse and Sasse, 1998). Tomato plants treated with 24-epibrassinolide showed an enhanced tolerance to high temperature due to inducement of the expression of mitochondrial small heat shock proteins, which possibly induced thermo tolerance (Singh and Shono, 2005). BRs induced salt tolerance in rice also by enhancing metabolic turn over of nucleic acids, soluble

proteins, and nitrate reductase activity (Anuradha and Rao, 2001; Anuradha and Rao, 2003).



IR28 **MTL119**

Figure 26. Influence of 24-epibrassinolide on salinity tolerance of 90 day-old plants (60 days after salt-stress) of the salt-sensitive cultivar IR28 and the salt-tolerant cultivar MTL119 under 100 mmol NaCl conditions

Furthermore, the results show that a higher salt concentration lead to a higher proline accumulation (see Session 3.3.2, Table 20, Table 21). This phenomenon can be explained by the following fact. At seedling stage, the rice plants were cultured in a hydroponic medium, thus the roots directly took up sodium and/or other ions from the nutrient solution. At tillering and heading stages then, plants were grown in soil pots, which were kept in trays with 3 cm level of salt solution,

thus they absorbed sodium and/or other ions from soil solution. Because of the absorption of ions through the soil solution, the salt concentration penetrating the roots and up to the plant is presumably lower than the salt concentration in the hydroponic medium. Also it should be considered that the plants at seedling stage are much smaller than at later growth stages. Therefore, the relative proline content measured in soil experiments was probably lower than contents measured in the hydroponic trial with the same salt concentration application. In addition, the salt stressed plants were one third smaller than the normal plants in the same seedling stage. For this reason it was also necessary to pool more salt stressed plants together with one sample for obtaining the required sample volume for proline assay, whereas fewer samples were needed for the control plants that posed a higher biomass. Thus, this could additionally be a reason for higher proline contents in stressed leaves of both varieties than proline contents of the non-stressed leaves in comparison with other experiments at tillering or heading stage.

In the current experiment, with 100 mM salt-stress applied to 30 day-old soil grown plants, plants were seriously injured by salinity, but the EBI treated plants were able to survive this conditions for 60 days and were even able to form panicles and to head, whereas most of the EBI untreated plants died (Fig. 26). EBI induced an enhanced tolerance to salinity in these rice plants, which is presumably based on the increase of proline content reducing the damage of salt on the salt-sensitive IR28 cultivars to a non-lethal level.

Thus, EBI promotes the proline accumulation in the salt-sensitive variety in many different growth stages, like seedling, tillering or heading stage. Presumably, in the experiments EBI was demonstrated to induce saline stress tolerance in rice. The adaptation mechanism to salt stress is hypothesized to be established on proline synthesis as a membrane stabilizer. By delivering additional OH-groups instead of the rising one of reduced water under stress, proline is presumed to protect membranes against damaging salt effects.

4.3. Effects of 24-epibrassinolide application on the yield and yield parameters of salinity affected rice

In normal conditions, one panicle is headed from the tip node of each mature tiller of the rice plant. It is the, so called, primary panicle. Under unfavorable environmental circumstances like super optimal nitrogen supply or cutting, lodging, secondary panicles will additionally emerge from the base node of the tiller. A tiller has only one primary panicle but it can have more than one secondary panicle.

The results in Session 3.3.3 and 3.4.3 show that EBl treatment increases the number of secondary panicles in rice. Although this effect is not necessarily a positive benefit for grain yield it indicates at least another role of EBl in plant developmental regulations. The mechanism may be explained by the regulation of some phytohormones. The quiescent axillary buds may be regarded as “replacement apices”, which remain inhibited under unfavorable conditions such as nutrient deficiency, drought or shading. However, the buds may be induced to develop into lateral branches in case the plant is exposed to favorable conditions, or the shoot apex is lost (Tamas, 1995). In oats, the emergence of the inflorescence releases the lateral buds from inhibition and allows them to develop into tillers (Harrison and Kaufman, 1980). Thus, the quiescent axillary buds or inhibition of secondary panicles in plants due to some above reasons should be discussed, and this phenomenon is regulated by phytohormones such as auxin, cytokinin. Apical dominance is stimulated by auxin and lateral buds are enhanced by cytokinin (Tamas, 1995).

According to the mechanism described above, in the experiments the apical dominance decreased after emergence of primary panicles. EBl was suggested to be able to induce the activity of cytokinin, thereby, increasing the secondary panicles of the EBI treated rice plants.

Cytokinins are known to stimulate lateral branching in several plant species, like BRs stimulated shoot proliferation, through stem elongation, but especially through an increase of lateral branching, which resulted in enhanced multiplication rate for the marubakaido apple rootstock. Like cytokinins, BRs have also been reported to

be involved on branching responses and changing endogenous cytokinin levels in various plant species (Pereira-Netto *et al.,* 2003).

Although secondary panicles do not contribute to the grain yield due to their immaturity at harvest time, this result may be interesting for the second crop (ratoon) in rice. It can increase the number of panicles and shorten the growth duration of the second crop. For example, in the flooded regions of the Mekong Delta of Vietnam, there are two rice crops per year normally. However, sometimes three rice crops per year are conducted but at a high risk to loose the second crop because of the annual flood which is usually arriving at the harvest time of this second crop. Therefore, it would be necessary to shorten the time for the second crop in order to avoid the flood. Some cultural practices are often applied such as using the very short growth duration variety (less than 90 days) or applying the ratoon crop from stocks of the first crop. The ratoon crop is popular nowadays. However, the high grain yield crop can be only obtained, if the stocks of the first crop have to sprout more new healthy tillers after harvesting. From the result of this experiment it is suggested that EBI application can satisfy these demands.

In rice cultivation, the grain yield is the final result of the whole farming process and the most important criterion in yield evaluation. The grain yield is based on the four main components: number of plants per area unit, number of panicles per plant, the number of filled grains per panicles, and the average weight of 1000 grains. In this experiment, as the plant density of all treatments was the same (23.5 plants m^{-2}), the analysis of grain yield was based on the grain yield per plant. The three main yield components, i.e., the number of panicles per plant, the filled grains per panicles and the average weight of 1000 grains, were measured and analyzed.

The formation of primary panicles of rice depends strongly on genotypes and environmental conditions. However, EBI did not affect the primary panicles of both varieties under different salt-stress applications in this experiment.

EBI did not affect the number of filled grains per panicle (FGPP) of the tested cultivars under non-stressed conditions, but induced more secondary panicles and also an earlier appearance of these compared to EBI untreated plants. Therefore, these secondary panicles can affect yield components, because they compete for

nutrient with the primary panicles (nutrient partition) and reduce the total grain yield by this way. Concurrently, EBI could not increase the number of FGPP of both cultivars after a three week salt-stress at the tillering stage. Perhaps, after a longer time under saline conditions, the plants were strongly injured and could not be recovered. As a result, the number of FGPP could not be improved. However, EBI significantly increased the number of FGPP of MTL119 but did not affect IR28 (Session 3.4.3) due to one week of salt stress simulated during heading time.
As MTL119 is the salt-tolerant variety, it was injured only slightly by one week of salt-stress, and EBI application was able to strengthen MTL119 to such a point that it could overcome this slightly salt-stress damage. As found by Ikekawa (1991), brassinosteroids are known as phytohormones to overcome stress symptoms. The relative effects of brassinosteroids are low when the cultivating conditions are generally favorable.

Size of grain is one of the characteristics of rice, which strongly depends on genotypes and environmental conditions. Similar to FGPP, under low salt stress levels ($\leq$ 50mM) at heading stage EBI application resulted in an avoidance of grain size loss in the salt tolerant MTL119 genotype. Under more saline conditions at tillering stage both cultivars lost grain size also when treated with EBI.

The reasons why grain size was reduced after EBI treatment under non-stress condition are probably identified with those for FGPP. The enhancing of secondary panicles started competition for nutrient between primary and secondary panicle.
Thus, EBI can rescue the grain size depletion caused by moderate salt stress but only under low level of stress (50mM NaCl) or the late growth stage (after heading). However, EBI has negative effects on grain size of rice under non-stress conditions.

Grain yield is a final result based on yield components. The treatment with EBI increased grain yield of both varieties. This effect, however, was limited to moderately saline conditions occurring during the vegetative growth stage and was more pronounced in salinity tolerant than in salinity sensitive cultivars.

The application of EBI did not influence the yield of rice under non-saline conditions, at very high salinity levels (100mM), or in situations when salinity occurred during the early growth stages (seedling salinity). This confirms that EBI may be effective reducing the damage of moderate salt-stress affected crops. Similar to the results of other studies, the relative effects of BRs are low when the conditions under which plants are growing are generally favorable. For example, positive effects of BRs were obtained on wheat grown under drought conditions, where as these effects minimized under regular conditions (Khripach *et al.*, 2000). BRs application results in cereals crop yield increase by 5 -20% depending on cultivars, climatic conditions, type of soil and level of applied fertilizers, mode and time of treatment (Khripach *et al.*, 2000).

In summary, the application of the brassinosteroide EBI can increase the grain yield of rice grown under moderate salt-stress conditions. The physiological mechanism for this enhanced stress tolerance has been shown to involve the accumulation of proline in the cell of leaves. Further studies are needed to confirm the positive effect of EBI application on salt stress tolerance and the positive mechanism of proline accumulation with a wider range of rice germplasm. The reported effects of EBI on rice further warrant to be tested in salinity affected rice-growing environments of the Mekong Delta.

5. CONCLUSIONS and RESEARCH NEEDS

The presented research findings confirm that treating seeds and leaves with the brassinosteroid 24-epibrassinolide (EBI) can positively affect the salt stress tolerance of rice in various development stages. These effects depend on the level of salinity tolerance of the genotype and the intensity and duration of the salt stress. Seed treatment with EBI promoted germination and the development of secondary roots. Foliar application tended to enhance tiller numbers and the leaf area index and significantly increased the grain yield under conditions of moderate salinity. In case of the salinity tolerant rice variety, this EBI-induced yield increase was explained by a higher number of filled spikelets and a larger grain size. All these effects were generally limited to conditions of mild salinity or to the salinity-tolerant genotype. The brassinosteroids were not effective under conditions of severe salt stress with high salt concentrations or the sensitive genotype. Any positive effect of EBI application on salinity tolerance in rice was associated with an increase in the concentration of proline in leaf tissues. The application of brassinosteroids is seen to be a promising complementary strategy in the efforts to minimize the negative effects of salinity on rice production.

The physiological mechanism for the observed enhanced stress tolerance has been shown to involve the accumulation of proline in plant tissues. Further studies are needed to confirm the positive effect of EBI application on the salt stress tolerance of rice and the role of proline accumulation. Also other possible physiological plant adaptation strategies involving both osmotic adjustment and the control of oxidative stress need to be investigated. The current study was limited to two rice genotypes and only two salinity levels applied under controlled conditions. Given the diversity of morphological and physiological adaptation strategies to salt stress, a much larger number of rice genotypes needs to be evaluated regarding their response to EBI application. The crop response also needs to be tested in field studies involving more diverse environmental conditions, possible season effects and a wider range of salinity levels.

REFERENCES

Abdullahi, B.A., Gu, X-G., Gan, Q-L. and Yang, Y-H (2003). "Brassinolide amelioration of aluminum toxicity in mungbean seedling growth." Journal of Plant Nutrition **26**(9): 1725-1734.

Akbar, M. and Ponnamperuma, F.N. (1982). "Saline soil of South and Southeast Asia as potential rice lands. In: IRRI, ed. Rice Research Strategies for the Future." IRRI, Manila, the Philippines: pp. 256–281.

Arnon, D.I. (1949). "Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*." Plant Physiology **24**: 1-15.

Anon (2002). "In: Hayat, S. and Ahmad A. (2003) eds. “Brassinosteroids: Bioactivity and crops productivity.” " Kluwer Academic Publishers, The Netherlands: p. 207.

Anuradha, S. and Rao, S.S.R. (2001). " Effect of brassinosteroids on salinity stress induced inhibition of seed germination and seedling growth of rice (*Oryza sativa* L.)." Plant Growth Regulation **33**: 151-153.

Anuradha, S. and Rao, S.S.R. (2003). "Application of brassinosteroids to rice seeds (*Oryza sativa* L.) reduced the impact of salt stress on growth, prevented photosynthetic pigment loss and increased nitrate reductase activity." Plant Growth Regulation **40**: 29-32.

Arteca, R.N. (1995). "Plant hormones: physiology, biochemistry and molecular biology." Kluwer Publisher, The Netherlands: pp. 206-212.

Asch, F., Dingkuln, M., Dörffling, K. and Miezan, K. (2000) Leaf K/Na ratio predicts salinity induced yield loss in irrigated rice. Euphytica **113**: 109-118.

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

Beaudoin, N., Serizet, C., Gost, F. and Giraudat, J. (2000). "Interactions between abscisic acid and ethylene signaling cascades." The Plant Cell **12**: 1103-1115.

Binzel, M.L., Hasegawa, P.M., Rhodes, D., Handa, S., Handa, A. K. and Bressan, R.A. (1987). "Solute accumulation in tobacco cells adapted to NaCl." Plant Physiology **84**(4): 1408–1415.

Bui, C.B. and Nguyen, T.L. (2004). "Improving rice productivity under water constraints in the Mekong Delta, Vietnam. In: Seng, V., Craswell, E., Fukai, S. and Fisher, K. eds. Water in agriculture." ACIAR Proceedings **116e**: 196-202.

Castillo, E., To, P.T., Huynh, T.T.T., Thai, N.H.T. and Tran, T.K.P. (2003). "Phenological and physiological responses of a rice cultivar to level and timing of salinity stress. In: Preston, N. and Clayton, H. eds. Rice-shrimp farming in the Mekong Delta: biophysical and socioeconomic issues." ACIAR Technical Report **52e**: 89-101.

Castle, J., Montoya, T. and Bishop, G.J. (2003). "Selected physiological responses of brassinosteroids: a historical approach. In: Hayat, S. and Ahmad, A. eds. Brassinosteroids: bioactivity and crops productivity." Kluwer Academic Publishers, The Netherlands: pp. 45-68.

Chen, J.-G., Pandey, S., Huang, J., Alonso, J.M., Ecker, J.R., Assmann, S.M. and Jones, A.M. (2004). "GCR1 can act independently of heterotrimeric G-protein in response to brassinosteroids and gibberellins in Arabidopsis seed germination." Plant Physiology **135**: 907-915.

Clouse, S.D. (1996). "Molecular genetic studies confirm the role of brassinosteroids in plant growth and development." The Plant Journal **10**(1): 1-8.

Clouse, S.D. and Sasse, J.M. (1998). " Brassinostroids: essential regulators of plant growth and development." Annual Review of Plant Physiology and Plant Molecular Biology **49**: 427- 451.

Clouse, S.D. and Zurek, D. (1991). "Molecular analysis of brassinolide action in plant growth and development. In: Cutler, H.G., Yokota, T. and Adam, G. eds. “Brassinosteroids: chemistry, bioactivity and applications.” American Chemical Society, Washington, DC: pp. 122-140.

Clouse, S.D., Langford, M., McMorris, T.C. and Baker, M.E. (1993). "Physiological and molecular effects of brassinosteroids on *Arabidopsis thaliana*." Journal of Plant Growth Regulation **12**: 61-66.

Debeaujon, I. and Koornneef, M. (2000). "Gibberellin requirement for Arabidopsis seed germination is determined both by testa characteristics and embryonic abscisic acid." Plant Physiology **122**: 415-424.

FAO (2000). "Extent and causes of salt-affected soils in participating countries." http://www.fao.org/ag/agl/agll/spush/topic2.htm

FAO (2001). "Lecture notes on the major soils of the world." http://www.fao.org/documents/show_cdr.asp?url_file=/DOCREP/003/Y1899E/y1899e09.htm

Fariduddin, Q., Ahmad, A. and Hayat, S. (2003). "Photosynthetic response of *Vigna radiata* to pre-sowing seed treatment with 28-homobrassinolide." Photosynthetica **41**(2): 307-310.

Fischer, K.S. (1996). "Improving cereals for the variable rainfed system: from understanding to manipulation. In: Singh, V.P., Singh, R.K., Singh, B.B., Zeigler, R.S. eds. “Physiology of stress tolerance in rice”." IRRI, Manila, the Philippines: pp. 1-9.

Flowers, T.J. and Yeo, A.R. (1981). "Variability in the resistance of sodium chloride salinity within rice (*Oryza sativa* L.)." New Phytologist **88**: 363-373.

Friebe, A., Volz, A., Schmidt, J., Voigt, B., Adam, G. and Schnabl, H. (1999). 24-episecasterone and 24-epicastasterone form *Lychnis viscaria* seeds. Phytochemistry **52**: 1607-1610.

Fujii, S. and Saka, H. (1992). "Effect of brassinolide on translocation of assimilate in rice plants during the ripening stage." Japan Journal of Crop Science **62**: 193-199.

Fujii, S. and Saka, H. (2001). "Distribution of assimilates to each organ in rice plants exposed to low temperature at ripening stage and effect of brassinolide on the distribution." Plant Production Science **4**: 136-143.

Fukuda, H. (1997). "Tracheary element differentiation." Plant Cell **9**: 1147-1156.

Girousse, C., Bournoville, R. and Bonnemain, J-L. (1996). "Water deficit-induced changes in concentrations in proline and some other amino acid in the phloem sap of alfalfa." Plant Physiology **111**: 109-113.

Grove, M.D., Spencer, G.F., Rohwedder, W.K., Mandava, N., Worley,J.F., Warthen J.D.J.R., Steffens, G.L., Flippen-Anderson, J.L. and Cook, J.C.J.R. (1979). "Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus* pollen." Nature **281**: 216-217.

Gu, X.-Y., Chen, Z-X. and Foley, M. E. (2003). "Inheritance of seed dormancy in weedy rice." Crop Science **43**: 835-843

Hamada, K. (1986). In: Sakurai, A., Yokota, T., Clouse, S.D. (1999). eds. Brassinosteroids streroidal plant hormones." Springer, Tokyo: pp. 137-161.

Harrison, M.A. and Kaufmann, P.B. (1980). "Hormonal regulation of lateral bud (tiller) release in oats (*Avena sativa* L.)." Plant Physiology **6**: 1123-1127.

Hirai. (1991). "In: Khripach, V.A, Zhabinskii, V.N. and de Groot, A.E. (1999) eds. Brassinosteroids: a new class of plant hormones." San Diego Academic Press: pp. 325-346.

Ikekawa, N. and Zhao, Y.J. (1991). "Application of 24-epibrassinolide in agriculture. In: Cutler, H.G., Yokota, T., Adam, G., eds. “Brassinosteroids: chemistry,

bioactivity, and applications."" American Chemical Society, Washington: pp. 280-291.

IRRI (2002). "Standard evaluation system for rice (SES)." IRRI, Manila, the Philippines: p.38

Kamuro, Y. and Takatsuto, S. (1999). "Practical application of brassinosteroids in agricultural fields. In: Sakurai, A., Yokota, T. and Clouse, S.D., eds. Brassinosteroids: steroidal plant hormones." Springer, Tokyo: pp. 223-241.

Khatun, S. and Flowers, T.J. (1995a). "The estimation of pollen viability in rice." Journal of Experimental Botany **46**: 151-154.

Khatun, S. and Flowers, T.J. (1995b). " Effects of salinity on seed set in rice." Plant, Cell and Environment **18**: 61-67.

Khripach, V.A., Zhabinskii, V.N. and De Groot, A.E. (1999). "Brassinosteroids: a new class of plant hormones." San Diego Academic Press: pp. 328-337

Khripach, V.A., Zhabinskii, V.N. and De Groot, A.E. (2000). "Twenty years of brassinosteroids: Steroidal plant hormones warrant better crops for the XXI century." Annals of Botany **86**: 441-447.

Khripach, V.A., Zhabinskii, V.N. and Khripach, N.B. (2003). "New practical aspects of brassinosteroids and results of their ten-year agricultural use in Russia and Belarus. In: Hayat, S. and Ahmad, A. eds. Brassinosteroids: bioactivity and crops productivity" Kluwer Academic Publishers, The Netherlands: pp. 189-230.

Koning, R.E. (1994). "Seeds and seed germination." Plant Physiology Information website.
http://plantphys.info/plants_human/seedgerm.html (7-18-3906)

Krishna, P. (2003). "Brassinosteroid-mediated stress responses." Journal of Plant Growth Regulation **22**: 289-297.

Krishnan, S., Azhakanandam, K., Ebenezer, G.A.I., Samson, N.P. and Dayanandan, P. (1999). "Brassinosteroids and benzylaminopurine increase yield in IR50 indica rice." Current Science **76**(2): 145-146.

Lafitte, H.R., Ismail, A. and Bennett, J. (2004) "Abiotic stress tolerance in rice for Asia: progress and the future". In: "New directions for a diverse planet". Proceedings of the 4th International Crop Science Congress, 26 Sep – 1 Oct 2004, Brisbane, Australia. Published on CDROM: 11-17.
Web site: www.cropscience.org.au

Le, H.N. (1999). "Biodiversity in the Mekong Delta." Community Biodiversity Development and Conversation Project, Cantho University. pp. 16.52.

Leubner-Metzger, G. (2001). "Brassinosteroids and gibberellins promote tobacco seed germination by distinct pathways." Planta **213**: 758-763.

Levitt, J. (1980). "Responses of plants to environmental stresses. V. II. Physiological ecology." Academic Press, New York: pp 365-384.

LingHwa, T. and Morishima, H. (1997). " Genetic characterization of weedy rices and the inference on their origins." Breeding Science **47**(2): 153-160.

Liu, J. and Zhu J-K. (1997). "Proline accumulatin and salt-stress-induced gene expression in a salt-hypersensitive mutant of Arabidopsis." Plant Physiology **114**: 591-596.

Lutts, S., Kinet, J.M. and Bouharmont, J. (1996a). "Effects of salt stress on growth, mineral nutrition and proline accumulation in relation to osmotic adjustment in rice (*Oryza sativa* L.) cultivars differing in salinity resistance." Plant Growth Regulation **19**: 207-218.

Lutts, S., Kinet, J.M., and Bouharmont, J. (1996b). "NaCl-induced senescene in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance." Annals of Botany **78**: 389-398.

Mandava, N.B. (1988). "Plant growth-promoting brassinosteroids. "Annual Review of Plant Physiology and Plant Molecular Biology **39**: 23–52.

MARD (2005). Vietnam News Agency May 25th, 2005.

Massoud, F.I. (1974). "Salinity and alkalinity as soil degradation hazard." FAO/UNDP Expert consultation on Soil Degradation (AGL:50/74/10), FAO, Rome: p. 21.

Mattioni, C., Lacerenza, N.G., Troccoli, A., De Leonardis, A.M. and Di Fonzo, N. (1997). "Water and salt stress-induced alterations in proline metabolism of *Triticum durum* seedlings." Physiologia Plantarum **101**: 787-792. .

Mohan, M., Madhan, S., Narayanan, L. and Ibrahim, S.M. (2000). "Chlorophyll stability index (CSI): its impact on salt tolerance in rice." IRRI, the Philippines. Crop Management and Physiology **25**(2): 38-39.
http://www.irri.org/publications/irrn/pdfs/vol25no2/IRRN25-2Tablecontents.pdf

MRC (2002). "Annual Report 2001." http://www.mrcmekong.org/annual_report/annual_report.htm.

MRC (2005). "Overview of the hydrology of the Mekong Basin." Mekong River Commission, Vientiane: p. 56.

Nguyen, N.D., Le, X.T. and Pham, T.P. (2003). "Rice-shrimp farming systems in the Mekong Delta: biological and socioeconomic issues." ACIAR Technical Report **52e**: 53-69.
Nguyen, T.C. and Nguyen, T.L. (2004). "Identification of rice genotypes adapted to adverse soils in Mekong Delta." OMONRICE **12**: 154-156. http://www.clrri.org/lib/omonrice/12-20.pdf.

Nguyen, V.S., Vo-Tong, X., and Tran, A.P. (1998). "History and future of farming systems in the Mekong Delta. In: Vo-Tong, X. and Matsui, S. eds. Development of farming systems in the Mekong Delta of Vietnam." Ho Chi Minh City Publishing House, Vietnam: pp.17-80.

Oldeman (1991). "http://www.fao.org/AG/AGL/agll/spush/intro" (date: May 18, 2006)

Pahlich, E., Kerres, R. and Jäger, H-J. (1983). "Influence of water stress on the vacuole/extravacuole distribution of proline in protoplasts of *Nicotiana rustica*." Plant Physiology **72**: 590-591.

Paleg, L.G., Douglas, T.J., Daal, A.V. and Keech, D.V. (1981). "Proline, betaine and other organic solutes protect enzymes against heat inactivation." Australian Journal of Plant Physiology **8**: 107-114.

Pereira-Netto, A.B., Schaefer, S., Galagovsky, L.R. and Ramirez, J.A. (2003). "Brassinostroid-driven modulation of stem elongation and apical dominance: applications in micropropagation. In: Hayat, S. and Ahmad, A. eds. Brassinosteroids: bioactivity and crops productivity " Kluwer Academic Publishers, the Neitherlands: 129-157.

Pirogovskaya, G.V., Bogdevich, I.M., Naumova,G.V., Khripach, V.A., Azizbekyan, S.G. and Krul, L.P. (1996). "New forms of mineral fertilizers with additives of plant growth regulators." Proceedings of the Plant Growth Regulation Society of America **23**: 146-151.

Pollard, A. and Wyn Jones, R.G. (1979). "Enzyme activities in concentrated solutions of glycinebetaine and other solutes." Planta **144**(3): 291-298.

Ponnamperuma, F.N. (1994) Evaluation and improvement of lands for wetland rice production**.** In: Senadhira, D. ed. Rice and problem soils in South and Southeast Asia." IRRI, Manila, the Philippines. Discussion Paper Series No.4. pp. 3-19.

Ponnamperuma, F.N., Bandyopadhya, A.K. (1980). "Soil salinity as a constraint on food production in the humid tropics. In: Priorities for alleviating soil relatedconstraints to food production in the tropics." IRRI, Manila, the Philippines: pp. 203-216.

Prusakova, L.D., Chizhova, S.I. and Khripach, V.A. (1985). "In: S. Hayat and A. Ahmad (2003) eds. “Brassinosteroids: bioactivity and crops productivity.”" Kluwer Academic Publishers, The Netherlands: pp. 207.

Rao, S.S.R., Vardhini, B.V., Sujatha, E. and Anuradha, S. (2002). "Brassinosteroids – A new class of phytohormones." Current Science **82**(10): 1239-1245.

Raven, J.A. (1985). "Regulation of pH and generation of osmolarity in vascular plants: a cost benefit analysis in relation to efficiency of use of energy, nitrogen and water." New Phytologist **101**: 25-77.

Reid, J.B. and Ross, J.J. (1995). "Intemodes length in *Pisum*. Two further gibberellin-insensitivity genes, lka and lkb. " Physiologia Plantum **75**: 81-88.

Richter, K. and Koolman, J. (1991). "Antiecdysteroid effects of brassinosteroids in insect. In: Cutler, H:G:, Yokota, T. and Adam, G. eds. Brassinosteroids chemistry, bioactivity and applications.” American Chemical Society, Washington: pp. 265-279.

Roth, U., Friebe, A. and Schnabl, H. (2000). "Resistance induction in plants by a brassinosteroid-containing extract of *Lychnis viscaria* L." Zeitschrift für Naturforschung **55c**: 552-559. .

Sairam, R.K. (1994). "Effect of homobrassinolide application on metabolism and grain yield under irrigated and moisture-stress conditions of two wheat varieties." Plant Growth Regulation **14**: 173-181.

Santarius, K.A. (1994). "Apoplasmic water fractions and osmotic potentials at full turgidity of some Bryidae." Planta **1993**(1): 32-37.

Santoro, M.M., Liu, Y., Khan, S. M.A., Hou, L-X. and Bolen, D.W. (1992). "Increased thermal stability of proteins in the presence of naturally occurring osmolytes." Biochemistry **31**: 5278-5283.

Sasse, J. (1999). "Physiological actions of brassinosteroids. In: Sakurai, A., Yokota,T. and Clouse, S.D. eds. Brassinosteroids: steroidal plant hormones." Springer, Tokyo: pp. 137-160.

Sasse, J.M., Smith, R. and Hudson, I. (1995). "Effect of 24-epibrassinolide on germination of seeds of *Eucalyptus camaldulensis* in saline conditions." Proceedings of the Plant Growth Regulation Society of America **22**: 136-141.

Senadhira, D. (1994). "Rice and problem soils in South and Southeast Asia." IRRI, Manila, the Philippines. Discussion Paper Series No. 4: pp. 1-186.

Schnabl, H., Roth, U. and Friebe, A. (2001). "Brassinostroid-induced stress tolerances of plants." Recent Research Development in Phytochemistry **5**: 169-183.

Shimose, N. (1995). "Salt and acid sulfate injuries. In: Matsuo, T., Kumazawa, K., Ishii, R., Ishihara, K. and Hirata, H. eds. Science of the rice plant." Food and Agriculture Policy Research Center, Tokyo V.2: pp. 983-991.

Singh,V.P., Neue, H.U. and Akbar, M.(1994) "Coastal saline soils for rice cultivation. In: Senadhira, D. eds. Rice and problem soils in South and Southeast Asia." IRRI, Manila, The Philippines. Discussion Paper Series No.4. pp. 20-35

Singh, I. and Shono, M. (2005). "Physiological and molecular effects of 24-epibrassinolide, a brassinosteroid on thermotolerance of tomato." Plant Growth Regulation **47**: 111-119.

Skriver, K. and Mundy, J (1990). "Gene expression in response to abscisic acid and osmotic stress." Plant Cell **2**(6): 503-512.

Steber, C.M. and McCourt. P. (2001). "A Role for brassinosteroids in germination in Arabidopsis." Plant Physiology **125**: 763-769

Suge, H. (1996). "Reproductive development of higher plants as influenced by brassinolide." Plant Cell Physiology **27**: 199-205.

Suh, H. S., Sato, Y.I. and Morishima, H. (1997). "Genetic characterization of weedy rice (Oryza sativa L) based on morpho-physiology, isozymes and RAPD markers." Theoretical and Applied Genetics **94**(34): 316-321.

Takatsuto, S. (1994). " Brassinosteroids: distribution in plants, bioassays and microanalysis by gas chromatography-mass spectrometry." Journal of Chromatography **658**: 3-15

Takematsu, T. and Takeuchi, Y. (1989). "In: Hayat, S. and Ahmad, A. eds. “Brassinosteroids: bioactivity and crops productivity.”" Kluwer Academic Publishers, the Neitherlands: pp.159-170.

Takematsu, T., Takeuchi, Y. and Choi, C.D. (1986). "In: Sakurai, A., Yokota, T. and Clouse, S.D. eds. Brassinosteroids: Steroidal Plant Hormones." Springer, Tokyo: pp. 137-161.

Takeuchi, Y., Omigawa, Y., Ogasawara, M., Yoneyama, K., Konnai, M. and Worsham, A.D. (1995). "Effects of brassinosteroids on conditioning and germination of clover broomrape (*Orobanche minor*) seeds." Plant Growth Regulation **16**: 153-160.

Tamas, I.A. (1995). "Hormonal regulation of apical dominance. In: Davies, P.J. ed. Plant hormones physiology, biochemistry and molecular biology " Kluwer Academic, the Neitherlands: pp. 572-597.

Tester, M. and Davenport, R. (2003). "Na+ tolerance and Na+ transport in higher plants." Annals of Botany **91**: 503-527.

Torell, M. and Salamanca, A.M. (2003). "Wetlands management in Vietnam's Mekong Delta: An overview of pressures and responses." WorldFish Center, Malaysia. pp. 1-19.

Tran, T.B. (1994). "Sustainability of rice-shrimp farming system in a brackish water area in the Mekong Delta of Vietnam. M.Sc.(Hons) thesis in systems agriculture." School of Agriculture and Rural Development, University of Western Sydney-Hawkesbury, N.S.W., Australia: pp. iii-iv.
http://library.uws.edu.au/adt-NUWS/public/adt-NUWS20030808.142159/index.html

Tuong, N.T. (2001). "Sea level measurement and sea level rise in Vietnam." PSMSL Report for Vietnam, Proudman Oceanographic Laboratory, Birkenhead, U.K.
http://www.pol.ac.uk/psmsl/reports.national+regional/vietnam/vietnam_2001.doc

Ueono, T., Adachi, A., Hamada, K., Nishi, S., Fujita, F. and Fujiwara, S. (1985). "In: Khripach, V.A., Zhabinskii, V.N., and de Groot, A.E. (1999) eds. Brassinosteroids: a new class of plant hormones." San Diego Academic Press: p. 224.

Vardhini, B.V. and Rao, S.S.R. (2003). "Amelioration of osmotic stress by brassinosteroids on seed germination and seedling growth of three varieties of sorghum." Plant Growth Regulation **41**: 25-31.

Vo-Tong, X. (1993). "Recent advances in integrated land uses on acid sulphate soils. In: Dent, D.L. and Van Mensvoort, M.E.F. eds. Selected papers of the Ho Chi Minh City symposium on acid sulfate soils." International Institute Land Reclamation and Improvement, Wageningen **53**: 129-136.

Vo, Q.M. (1995). "Use of soil and agrohydrological characteristics in developing technology extrapolation methodology: a case study of the Mekong Delta, Vietnam. Msc thesis, University of the Philippines."

Wang, S.G. (1997). "Influence of brassinosteroid on rice seedling growth" International Rice Research Notes **22**: 20-21.

Wang, S. G. and Deng, R.F. (1992). "In: Hayat, S. and Ahmad, A. eds. Brassinosteroids: bioactivity and crops productivity." Kluwer Academic Publishers, The Netherlands: pp. 231-246.

Wang, T.-W., Cosgrove, D.J. and Arteca, R.N. (1993). "Barssinosteroid stimulation of hypocotyls elongation and wall relaxation in Pakchoi (*Brassica chinensis* cv Lei-Choi)." Plant Physiology **101**: 965-968. .

Wassmann, R., Nguyen, X.H., Chu, T.H. and To, P.T. (2004). "Sea level rise affecting the Vietnamese Mekong Delta: water elevation in the flood season and implications for rice production." Climatic change **66**: 89-107.

White, I., Melville, M. and Sammut, J. (1996). "Possible impacts of saline water intrusion floodgates in Vietnam's Lower Mekong Delta." Seminar on environment and development in Vietnam. AusAID, International Seminar Support Scheme. http://coombs.anu.edu.au/~vern/env_dev/papers/pap07.html (date 20/06/2006)

Xiong, L., Schumaker, K.S. and Zhu, J-K. (2002). "Cell signaling during cold, drought and salt stress." Plant Cell **(supplement 2002)**: 165-183.

Yamaguchi, T., Wakizuka, T., Hirai, K., Fujii, S. and Fujita, A. (1987). "Stimulation of germination in aged rice seeds by pretreatment with brassinolide." Proceeding of Plant Growth Regulation Society of America **14**: 26-27.

Yokota, T. (1997). "The structure, biosynthesis and function of brassinosteroids." Trends Plant Science **2**: 137-143.

Yokota, T. (1999). "The history of Brassinosteoids: discovery to isolation of biosynthesis and signal transduction mutants. In: Sakurai, A., Yokota, T., Clouse, S.D. eds. Brassinosteroids: steroidal plant hormones." Springer, Tokyo: pp.1-20.

Yu, J.Q., Huang, L.F., Hu, W.H., Zhou, Y.H., Mao, W.H., Ye, S.F. and Nogues, S. (2004). "A role for brassinosteroids in regulation of photosynthesis in cucumis sativus." Journal of Experimental Botany **55**(399): 1135-1143. .

Zhao, Y.J. and Chen, J. (2003). "Studies on physiological action and application of 24-Epibrassinolide in agriculture. In: Hayat, S. and Ahmad, A. eds. Brassinosteroids: bioactivity and crops productivity." Kluwer Academic Publishers, the Neitherlands: pp. 159-170.

Zhu, J.-K. (2001). "Plant salt tolerance." Trends in Plant Sciences **6**: 66-71.

Zurek, D.M., Rayle, D.L., McMorris, T.C. and Clouse, S.D. (1994). "Investigation of gene expression, growth kinetics and wall extensibility during brassinosteroid-regulated stem elongation." Plant physiology **104**: 505-513.

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Conferences

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Phap, V-A.; Cerboncini, C.; Brandt, A. ; Krause, J.; Drews, J.; Friebe, A. & Schnabl, H. (2006) Induction of salt tolerance by brassinosteroids in rice (*Oryza sativa*). Biotechnology Seminar 2006. BMBF. Poster.

Equipment

pH, EC measurers**,** pH/Cond 340i (WTW company, Germany)

Photosynthetic measurers

Gas exchange measurer (Ciras-1, England)

Fluorometer Imaging-PAM (Walz, Germany)

Spectrophotometer: Heλios α (Thermo Spectronic, England)

Growth chamber: MLR-3508H (Sanyo, Japan)

Soil Sterilizer: Sterilo (H. Nitsch + Sohn GmbH &Co., Germany)

Other equipment in the laboratory: desiccator Type UT6200, (Haraeus, Germany), grain-moisture (Protimeter, England)

Chemical

- 24-epibrassinolide, $C_{28}H_{48}O_6$ [(22R, 23R, 24R)-2α, 3α, 22, 23-tetrahydroxy-24-methyl-B-homo-7-oxa-5α-cholestane-6-one} (>95% pure)
  Weight of mole = 480.68g.
  Toxicity: acute oral toxicity for rat, LD50>2000mg/kg; mouse LD50>1000mg/kg. Acute percutaneous toxicity for rat LD50>2000mg/kg
  Product of CIDtech Research Inc. (USA)

- Chemicals for analyzing chlorophyll content: Acetone, HPLC grade 99.9%, (Roth Company, Germany)

- Chemicals for analyzing proline
  - Sulfosalicylic acid 95-97% (Merck company, Germany)
  - Nihydrin acid, >99% concentration (Roth company, Germany)

- Acetic acid, 100% (Merck company, Germany)
- Phosphoric acid, 99% (Merck company, Germany)
- Toluene, HPLC grade 99.7% (Alfa Aesar company, Germany)

o Nutrient solution for hydroponic plant culture according to Yoshida (1976).

Yoshida nutrient solution:

1. NH_4SO_3 91.4 g l^{-1}
2. $MgSO_4.7H_2O$ 324 g l^{-1}
3. $NaH_2PO_4.2H_2O$ 40.3 g l^{-1}
4. K_2SO_4 71.4 g l^{-1}
5. $CaCl_2.2H_2O$ 88.6 g l^{-1}
6. Micronutrient Solution (Yoshida, 1976)

Using 1,25 ml of each solution per litter and adjust to pH 5

o Fertilizer: N-P-K-S: 14-10-20-3, (Norsk Hydro company, Norway)