EFFECTS OF DIFFERENT CONCENTRATIONS OF BRASSINOSTEROIDS ON UPLAND RICE

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ABSTRACT

A field experiment was conducted at the rain shelter of Faculty of Sustainable Agriculture in Universiti Malaysia Sabah, Sandakan. This study is to investigate the effect of the different concentrations of Brassinosteoid on upland rice. The objectives of the study were to determine the brassinosteroid effect on the growth of upland rice and to determine the brassinosteroid effect on the yield of upland rice. The experimental design was CRD using 0 mg L^{-1} (control), 0.25 mg L^{-1}, 0.50 mg L^{-1}, 0.75 mg L^{-1} and 1.00 mg L^{-1} of brassinosteroid each replicated five times on Paddy Mutiara. Results were analysed by using Statistical Analysis Software (SAS). The application of brassinosteroid on day 130 shows that there are a significant on the chlorophyll content of the upland rice ($F (4, 24) = 4.07$, $p< 0.0142$) and the photosynthesis rate of upland rice ($F (4, 24) = 5.30$, $p< 0.0045$). The application of 1.00 mg L^{-1} is the best treatment. This experiment can be tried with other varieties of upland rice to find out if the results of this experiment are applicable to them.

KESAN KEPEKATAN BRASSINOSTEROIDS YANG BERBEZA-BEZA TERHADAP PADI HUMA

ABSTRAK

Satu kajian telah dijalankan di rumah teduhan hujan Fakulti Pertanian Lestari, Universiti Malaysia Sabah, Sandakan untuk mengkaji kesan kepekatan Brassinosteroids yang berbeza-beza terhadap padi huma. Objektif kajian ini adalah untuk menentukan kesan brassinosteroids terhadap pertumbuhan padi huma dan untuk menentukan kesan brassinosteroids terhadap hasil tuaian padi huma. Reka bentuk eksperimen ialah CRD dengan menggunakan 0 mg L^{-1} (kawalan), 0.25 mg L^{-1}, 0.50 mg L^{-1}, 0.75 mg L^{-1} dan 1.00 mg L^{-1} Brassinosteroids, di mana setiap rawatan direplikasikan sebanyak lima kali. Semburan brassinosteroid pada hari 130 menunjukkan ada perbezaan yang ketara terhadap kandungan klorofil padi huma ($F (4, 24) = 4.07$, rice $p< 0.0142$) dan kadar fotosintesis padi huma ($F (4, 24) = 5.30$, $p< 0.0045$). Penggunaan kepekatan brassinosteroids sebanyak 1.00 mg L^{-1} merupakan rawatan yang paling berkesan. Kajian ini boleh dicuba terhadap varieti padi huma yang lain untuk menglihat keberkesanannya.

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LIST OF UNITS, SYMBOLS, ABBREVIATIONS

ANOVA	Analysis of Variance
BR	Brassinosteroid
DOA	Department of Agriculture
FAO	Food and Agriculture Organisation
FPL	Fakulti Pertanian Lestari
IRRI	International Rice Research Institute
mg mL^{-1}	miligram per millilitre

CHAPTER 1

INTRODUCTION

1.1 Introcduction

The cultivated rice plant, *Oryza sativa,* is an annual crop of the Graminae family. There are two distinct types of domesticated rice, *Oryza sativa,* or an Asian rice and *Oryza glaberrima,* African rice that ate widely grown in the world. Rice varieties can be grouped Into two categories. The first category is the short-duration varieties which mature in 105-120 days and the second categories is the long-duration varieties which mature in 150 days. A 12-day variety spends 60 days in the vegetative phase, 30 days in the reproductive phase and 30 days in the ripening phase. *Oryza sativa*, or Asian rice, contains two major groups. The first group is indica (long-grain) and the second group is japonica (short-grain). Other types of Asian rice include glutinous rice and aromatic rice. The long-grain of indica varieties are grown in hot climates. The japonica varieties can be grown in both hot climate and temperate conditions.

In Malaysia, rice is normally cultivated either as upland rice or wet paddy. Rice plants take around three to six months to grow from seeds to mature plants. This is also depending on the rice variety and environmental condition. For example, upland rice has longer life period compared to wetland rice. Thus, this is probably why wetland rice is more preferred by the farmers. Upland rice cultivation is practised mostly by the rural communities living especially in Sabah and Sarawak (Hanafi *et al.*, 2009).

Rice is widely grown cereal crop ans the staple food for more than half the world's population. Global rice production has been able to meet population demands. Jowever, the rice crisis in June 2008 was an eye-opener for Asian countries especially, as it had greatly affect the main consumer if rice. Malaysia was badly affected by this crisis as the country's major exporting countries such as Vietnam imposed export bans or restriction to protect their domestic consumers. The rice supply was reduced in the world market, hence, increased the price rise. During this time, Malaysia immediately found itself unable to guarantee sufficient rice for the nation in the following three months. The food crisis exposed Malaysia's persistent and increasing food insecurity problem. This happened when rice production is not aligned with the consumption.

Recently, the demand for rice has increased sensationally as the population of Malaysian citizens grows. More than 28.7 million people in Malaysia depend on rice as their staple diet. This is ultimately the hardest challenge for government as they have to ensure the sufficient rice supply for the citizen. According to the National Agro-Food Policy (2011-2020), the first objective that needs to be achieved is to address food security and safety to ensure availability, affordability and accessibility. Self sufficient level (SSL) has to be maintained at 70%. Import of rice by country shows that our country brings in more rice from Vietnam (54.1%), followed by Thailand (19.3%), Pakistan (12.6%), Cambodia (9.6%), India (3.6%) and others (0.9%). The total import of rice is 1,583.8 thousand metric tones in 2013 where at the same time, Malaysia has produce 2,626,881 tons of rice. This shows the increase of Malaysia's rice production as in 2012, the rice production was 2,599.382 mt/ha (DOA, 2013).

Malaysia cultivates more wetland rice compared to upland rice. This is because, wetland rice gives more yield than upland rice. Wetland rice rely more on water supply. Thus, the dry season from May to October is the major problem for the wetland rice production. Upland rice does not rely much on water supply. However, adequate and assured soil moisture reserves during critical period of growth, regular rainfall during cropping season and fertile soil with low risk of erosion is the condition that needs to be fulfilled. Weed competition is more serious in upland rice than in wetland rice because upland fields do not have stagnant water to restrain weed growth. Poor rice germination due to dry season usually results in excessive weed growth. Some weeds in upland rice can resist drought better than the rice because their root go in deeper into the soil to absorb moisture. Increases in both carbon dioxide levels typically increase biomass production, but not necessarily yield. Higher temperature can decrease rice yields as they can make rice flowers sterile, meaning no grain is produced. Higher respiration losses linked to higher temperatures also make rice less productive (IFPRI, 2015). Flooding can be a big problem to the rice crop as it cannot survive submerged under water for a long period of time. The rising of sea level in coastal areas will cause flood to happen. The predicted increased intensity of tropical storms at speed of 39-73 mph, with climate change will likely obstruct rice production (Radzi and Awang, 2013). The monsoon season between October to January has greatly affecting the east coast states, which are the major rice producer in Malaysia.

Plants can adapt very fast to changes in their environment. Hormones, chemical messengers that are activated in direct response to light and temperature stimuli help them to achieve the changes. Plant steroid hormones, Brassinosterois are synthesized by plants and are powerful hormones influencing many aspects of plant growth. Brassinosteroid influence various developmental processes like seed germination, rhizogenesis, flowering, senescence, abscission, and maturation (Anuradha *et al.*, 2003). Brassinosteroids also confer resistance to plant against various abiotic stresses. Thus, application of brassinolide might help the rice to be immune to weed and pests' infections and increase the yield.

1.2 Justification

The cultivation of upland rice is unlikely favourable in Malaysia. Upland rice is grown aerobically in upland environments, mostly by rural communities in Sabah and Sarawak (Vincent, 2010). Research on upland rice has been neglected because of its low yield and unstable grain yields, even though itis widely grown in the interior parts of the country (Mariam *et al.*, 1991). Chen *et al.* (2012) claimed that application of different concentration of brassinosteroid on upland rice yield may provide important information on the ability of brassinosteroid to stimulate upland rice yield.

1.3 Objective

To determine the brassinosteroid effect on the growth of upland rice.

1.4 Hypothesis

Ho: There is no significant difference of the brassinosteroid effect on the growth of upland rice.

Ha: There is significant difference o of the brassinosteroid effect on the growth of upland rice.

CHAPTER 2

LITERATURE REVIEW

2.1 Rice Morphology

Rice plants are annual grass that belongs to the family *Graminae* and the genus *Oryza* are the only cereal plants that can be grown in upland area and flooded-soils area. The grown species of *Oryza sativa* can be grouped into three varietal types based on the morphological and physiological characteristics. The three varietal types are *indica, japonica* and *javanica*. These varietal types also differ in their geographical distribution (Panda, 2010). Rice plant is a diploid species having 24 chromosomes.

The parts of the rice plant can be divided into roots, stem and leaves, reproductive organs and grains. The root system of rice plant consists of numerous nodal roots that certainly affect the spatial distribution of the root system in the soil, which seems to relate to yield and lodging opposition (Abe and Morita, 1994). The fibrous roots of rice plant provide support and also absorb nutrients and water from the soil. The stem of rice plant transfer the nutrients absorbed by the roots to other parts of plant, at the same time provides support to the leaves and reproductive structures.

The leaves contain chlorophyll which is important for photosynthesis as it absorbs sunlight and utilizes its energy to synthesise carbohydrates from carbon dioxide and water. The angle of rice leaf inclination is an important trait of rice architecture as it affects the yield when it plays role in determining the photosynthetic efficiency and also involves in the light capture (Zhao *et al.*, 2010).

The reproductive organs of *Oryza sativa* can be differentiated into panicle and spikelet. The panicles known as flower cluster that result from the change of a vegetative apical meristem to a reproductive apical meristem. When the spikelet mature and develop into grains, the panicle will drop and no longer stands upward as when during the blooming stage. Each spikelet possesses a set of flower parts that consists of six stamens and a pistil. The fertilization occurs when the stigma catches pollen from the stamens and carried down to the ovary. Differences in spikelet number at harvest could be offset by differences in individual grain weight and percentage of filling spikelet (Sheehy *et al.*, 2001). Grain of rice plant is resulted from the fertilization of egg by pollen. The endosperm is the edible portion that contains starch, protein, fats, crude fibre and inorganic matter.

2.2 Growth Stages of Rice

The growth stages of rice can be divided into three stages. Those stages are vegetative stage, reproductive stage and ripening stage. The vegetative stage refers to a period from germination to the initiation of panicle primordial; the reproductive stage, from panicle primordial initiation to heading, and the ripening period, from heading to maturity (Panda, 2010).

The vegetative phase has four stages before going into the reproductive phase. Those four stages are seedling stage, transplanting stage, tillering stage and vegetative lag phase. A 120 days variety will spend 60 days in vegetative phase.

During seedling growth the plants establish by developing sufficient number of seminal roots and leaves, and then will terminate when the plants put forth tillers or branches (Panda, 2010). During transplanting stage, the secondary adventitious roots develop within four to ten days after transplanting. The tillering stage normally takes two to three weeks to complete. Rice plant is no longer producing tiller once the tertiary tillers produced. Vegetative lag phase is the period between maximum tillering and panicle initiation in which some of the tillers die because of tiller mortality (Panda, 2010).

The reproductive phase itself has two stages to be completed before going into ripening phase. The reproductive phase needs to undergo panicle initiation stage, internode elongation and booting stage, heading stage and flowering stage. During the reproductive stage of rice, the most severe water deficits tend to occur (O'Toole and

Garrity, 1994). The reproductive stage takes to 25 days to be completed. The panicle initiation stage occurs approximately from 21 to 25 days before heading but flowering primordium can be recognized only a week after initiation stage has occurred (Panda, 2010). The internode elongation usually begins around the initiation of panicle primordial and continues until heading. The developing panicle causing bulging in the leaf sheath and this indicates the booting stage has begun. The heading stage is indicated by percentage, where 50 percent heading stage takes place when 50 percent of rice panicle completely emerged from the stem. The flowering stage happens after booting where the spikelets are successfully blooming and followed by pollination and fertilization.

The ripening phase of rice is considered complete once the crop has passed through milky stage, dough stage and maturity stage. Milky stage occurs between seven to twelve days after anthesis where the watery content of the grain turn milky (Panda, 2010). The dough stage occurs as the milky substance turn into hard dough. This stage requires two to three weeks to be completed. The maturity stage is considered complete when the grains turn hard, clear and free from greenish hint.

The seed maturity can be classified into physiological maturity and harvest maturity. At physiological maturity, the rice panicles are completely mature at top and few grains at bottom at immature stage. An abscission layer forms between fruit and peduncle (Panda, 2010). During physical maturity, there is no further increase in dry matter in the economic part. Sudden fall of moisture content from 40 percent to 20 percent indicate the attaining of physiological maturity. Harvest maturity of rice generally occurs from seven to ten days after physiological maturity in rice. The loss of moisture is expected to occur during this stage.

2.3 Factors Influencing Growth and Development of Rice

2.3.1 Germination

Temperature, soil moisture, light, aeration and dormancy of seeds have a great effect on seed germination. High temperature inhibited germination by decreasing the abundance of proteins involved in methionine metabolism, amino acid biosynthesis, energy metabolism, reserve degradation, protein folding and stress responses (Liu *et al.*, 2013). High temperature sprouting is often used to strengthen rice seed germination, where in the contrary, low temperature treatment could injure its radicle (Howell *et al.,* 2007).

Rice seed germinate well within the moisture regime of field capacity to 50 percent available soil moisture (Panda, 2010). According to Ken-Ichi Matsushima and Jun-Ichi Sakagami in their study, the emergence time was reduced in priming seeds compared with that in untreated seeds because of increased shoot elongation with dry matter production under a wide range of soil moisture conditions, except flood (Sakagami and Matsushima, 2013).

Seed dormancy is defined as a physiological or physical condition of viable seed that prevent germination even in the presence of otherwise favourable germination conditions. Dormancy and modes of storage have maintained seeds viable for as long as 10,000 years. Seed dormancy is usually estimated by germination rate, but complex inheritance of seed dormancy has been noted by breeders and geneticists. According to E.H. Roberts on his study on dormancy of rice seed, harvesting the seed prematurely tends to speed up the process leading to loss of dormancy whereas late harvesting has little influence on the date on which the mean dormancy period is achieved (Roberts, 1960).

2.3.2 Seedling Growth

Salinity had significant effect on plumule and radicle fresh. According to the study performed by Kamyar Kazemi and Hamdollah Eskandari, the plumule and radicle length of seedlings grown in salt solutions showed decline, indicating that the salt stress not only affected germination but also the growth of seedlings, which indicates that synthetic ability of seed, and thus, dry matter production of the seedlings, was affected (Eskandari and Kazemi, 2011). According to Linghe Zeng and Michael C. Shannon, significant reduction of seedling growth occurred at higher cumulative thermal time than those seedlings at higher salt level, which indicates that plants affected by salts at low concentration can withstand salt stress for longer period of time but significant reduction of seedling growth occurs (Zeng and Shannon, 2000).

A temperature of 22°C or below is considered subnormal for seedling growth as the optimum level for seedling growth is at at 35°C, and will decline sharply if the temperature gets any higher (Krishnan, 2011). According to Kishnan, the long-term effects of high-temperature stress may include delayed germination or loss of vigour, leading to reduced emergence and seedling establishment.

2.3.3 Leaf Growth

Solar radiation, temperature, mineral nutrients and water status are important factors that decide the size of the leaf (Panda, 2010). Study conducted by Yoshihiro Hirooka shows the results indicated that the basal fertilizer application enhanced leaf growth, and the additional fertilizer mainly extended the period of enhancement, leading to a higher leaf area index (LAI) (Yoshihiro *et al.*, 2016).

The study about the influence of high water temperature on leaf growth in rice plant, *Oryza sativa,* shows that the high water temperature also decreased the total leaf length (blade and sheath) of (n)th to (n+3)th leaves and the blade area of (n+1)th to (n+3)th leaves (Sasaki, 2002).

The carbohydrate overly produced by the plant is immediately used in growth. This surplus is stored in various tissues and is utilized when the current supplies become inadequate to support the requirements of growth and respiration. At later stages, senescence sets in and less of chlorophyll occurs. Subsequently, organelles like plastids, endoplasmids reticulum, mitochondria and all membranes are disrupted, which then lead to gradual water loss and finally the leaf dies (Panda, 2010).

2.3.4 Grain

From the study conducted on the effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains, the results indicate that salinity affects photosynthesis through both its effects on stomatal closure and its interference with photosynthetic reactions in chloroplasts. They conclude that the marked harmful effect of salinity on the physiology and biochemical constituents of rice plants during the reproductive phase is closely associated with a lowered photosynthetic rate and a lower translocation of photosynthates, which is responsible for reduced grain growth (Sasaki, 2002).

Water stress or small assimilate supply during early panicle development has been shown to reduce grain number per panicle (Yoshihiro *et al.*, 2016). The study on effect of water stress during grain- filling period on rice grain yield and its quality under different nitrogen levels showed that when the plants were grown under normal N level, water stress markedly reduced the grain-filling percentage and grain weight, resulting in a significant decrease of grain yield by 11.6% to 14.7%.

2.4 Brassinosteroid

Brassinosteroids (Brs) are a class of plant polyhydroxysteroids that have been recognized as a new kind of phytohormones that play an essential role in plant development. Brassinosteroids are structurally related to animal and insect steroid hormones. Intensive research conducted on BRs reveals that they elicit a broad spectrum of physiological and morphological responses in plants, including stem elongation, leaf bending and epinasty, induction of ethylene biosynthesis and proton pump activation, synthesis of nucleic acid and proteins, regulation of carbohydrate assimilation and allocation, and activation of photosynthesis. Furthermore, BRs can protect plants from various biotic and abiotic stresses, such as soil salinity, high temperatures and heavy metals (Wu *et al.,* 2008)

Brassinosteroids are plant growth regulator that found at low concentrations throughout plant kingdom and are widely distributed in lower and high plants, which first was isolated from the pollen of *Brassica napus.* Then they have been detected in all plant organs such as anthers, seeds, leaves, stems, roots, flowers, and grains. They have been found to be biologically active in the various bioassay systems designed for gibberellins, auxins and cytokinins (Yakota *et al.,* 1990). The most abundant brassinosteroid is known as Brassinolide.

Brassinosteroids have known to promote or boost apical dominance, leaf senescence, enhance seed germination and gravitropism, stimulate the production of ethylene, inhibit root growth and formation of stomata, boost the formation of xylem, prevent premature abscission of fruit and increase the resistance to freezing where at the same time increase the yield of cereal crops (Reuzeau *et al.,* 2008)

2.4.1 Structure of Brassinosteroids

Brassinosteroids can be derived from the 5a-cholestane carbon skeleton that can be classified into four structural characteristics (Adam and Marco, 2002). The first structural characteristic is, ring A mono- to trioxygenated, always oxygenated at carbon 3. The second structural characteristic, ring B presenting a 6-oxo-7-oxalactone or a 6-keto function or saturation. All-trans -junctions of rings A – D, is the third structural characteristics. The fourth structural characteristics of Brassinosteroids is, 22a, 23a-dihydroxylated, mostly alkylated at carbon 24, sometimes methylated at carbon 25 and sometimes unsturated between carbons 24 and 28. Figure 2.1 shows the general formula of natural Brassinosteroids.

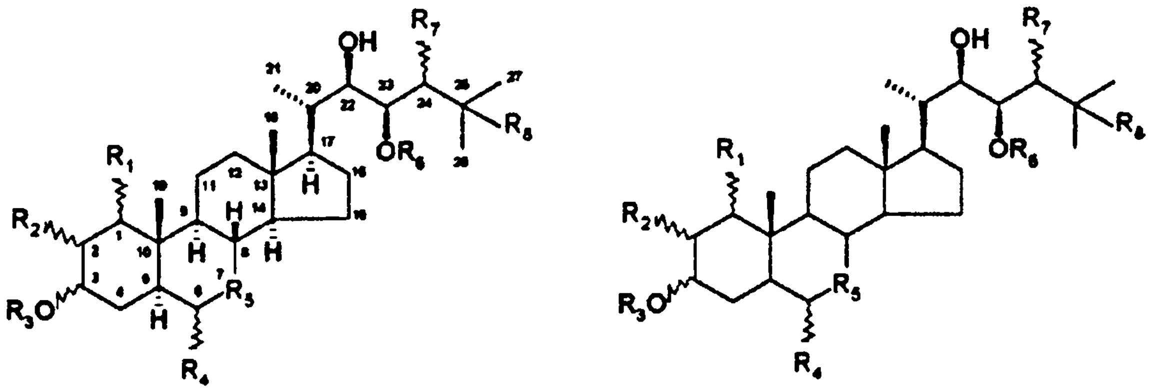

Figure 2.1 General formula of natural Brassinosteroids

Source: Adam and Marco, 2002

2.4.2 Metabolic Pathways of Brassinosteroids

According to Bajguz, and Tretyn (2003) the BR metabolic pathway can be classified into two types. First type is modification of the steroidal skeleton and second is modification of the side-chain.

The most common modification to the steroidal skeleton is esterification; to the side-chain is hydroxylation. Bioactive BRs can be inactivated by different processes, including dehydrogenation at the C-3 and C-23 positions, demethylation at the C-26 and C-28 positions, epimerization at the C-2, C-3 and C-24 positions, hydroxylation at the C-20, C-25 and C-26 positions, side-chain cleavage at the C-20/C-22 position and sulfonation at the C-22 position.

Active BRs are also changed into multiple forms by esterification at the C-3 position and glycosylation at the C-2, C-3, C-23, C-25 and C-26 positions. The conjugated compounds could serve as pool of inactive BRs that can be converted to active forms by de-conjugation reactions (Bajguz and Tretyn, 2003).

2.4.3 Brassinosteroids Signalling

The BR signal is transduced by a receptor kinase-mediated signal transduction pathway, which is distinct from animal steroid signalling systems. Recent studies have fully connected the BR signal transduction chain and have identified thousands of BR target genes, linking BR signalling to numerous cellular processes. Molecular links between BR and several other signalling pathways have also been identified (Zhu *et al.*, 2013).

Based on the rate of recent discoveries, it is highly likely that the major steps of BR signal transduction, from cell surface perception to the activation of specific nuclear genes, will be revealed during the next few years. The dramatic phenotype of BR-deficient and insensitive mutants is clear evidence of the critical importance of BR activity in the growth and development of plants (Clouse, 2011).

2.5 Application of Brassinosteroids on Rice

2.5.1 Promote Cell Elongation

Brassinosteroid (BR) works together with giberellin (GA) hormone to regulate plant cell elongation. The study conducted by Tong *et al.* (2014) showed that, under physiological conditions, BR boosts GA accumulation by regulating the expression of GA metabolic genes to stimulate cell elongation. This study showed that when excessive active BR is applied, the hormone mostly induces GA inactivation through up regulation of the GA inactivation gene GA2ox-3 and also represses BR biosynthesis, resulting in decreased hormone levels and growth inhibition. As a feedback mechanism, GA extensively inhibits BR biosynthesis and the BR response. Thus, GA treatment decreases the enlarged leaf angles in plants with enhanced BR biosynthesis or signalling.

BRs promoted the cell elongation of rice seedlings at a low temperature (15 °C). The effect of indole-3-acetic acid (IAA) on the cell elongation was markedly lowered, while the combination of BRs and IAA synergistically promoted the cell elongation (Bajguz and Tretyn, 2003).

Promotion of the second leaf sheath elongation, the characteristic response of dwarf rice varieties to gibberellins, was significantly but modestly enhanced by BR at a dosage of 10,000 ng/plant, five orders of magnitude higher than the minimal dosage response to GA3. Thus, they conclude that the BR-induced bending response may be mediated through endogenous auxin as IAA and α-naphthaleneacetic acid caused significant bending at 5,000 ng/plant, and both auxins significantly synergized the effect of BR on the bending, IAA being effective at 500 ng/ plant in this regard (Takeno and Pharis, 1982).

2.5.2 Seedling Growth

The study on the effect of salinity stress induced inhibition of seed germination and seedling growth of rice (*Oryza sativa L.*) was conducted. The result 6showed that the effect of 24-epibrassinolide and 28-homobrassinolide on the inhibition of germination and seedling growth of rice hindered by salinity stress. Brassinosteroids were found to reverse the inhibitory effect on germination and seedling growth. The activation of seedling growth by brassinosteroids under salinity stress was associated with enhanced levels of nucleic acids and soluble proteins (Anuradha and Rao, 2001).

According to Andrzej Bajguz and Shamsul Hayat, BRs removed the salinity-induced inhibition of seed germination and seedling growth in case of rice (*Oryza sativa*). BRs also restored the level of chlorophylls and increased nitrate reductase activity under salt stress. The activity of this enzyme plays a pivotal role in the supply of nitrogen and the growth and productivity of plants, especially in cereals. The reduced nitrate reductase activity in the leaves of salt-stressed plants is attributed to salinity inhibited nitrate transport to the shoot, which in turn is due to interference with nitrate uptake and xylem loading (Bajguz and Hayat, 2009).

2.5.3 Leaf Expansion

Leaf spraying of BRs on the rice seedlings at the fourth leaf stage increased plant height and the fresh weights of tops and roots under chilling stress. BR sprayed on the completely expanded 4th leaves did not increase their blade length, but that sprayed on the expanding 5th and 6th leave strikingly increased their blade length. These results are the evidence that BRs were more effective on the protective of old rice leaves against cold stress (Bajguz and Tretyn, 2003).

2.5.4 Yield

BRs affect many plant processes, including those that control tiller number, leaf size, and leaf angle (Chuan *et al.,* 2008). This suggests that manipulation of BR levels in specific parts of crop plants could be one way to further increase grain yields. Sakamoto (2008) suggested that, reduced BR levels make the leaves of rice plants more upright, allow planting at higher densities, and provide increases in grain yield per plot without a need for additional fertilizer.

The study on the effect of brassinosteroid and brassinosteroid mimic on photosynthetic efficiency and rice yield under heat stress showed that the paddy field applied by 1 nm EBR and/ or 1 nm DHECD at the reproductive stage during the hot season could increase the rice yield, especially, the number of filled seeds (Jutiporn *et al.,* 2015). The study on boosting crop yields with plant steroid shows the sterols and BRs play a role in promoting seed germination, both under normal and stress conditions. Therefore, modification of the sterol and BR contents in seeds might have important agronomical applications (Reuzeau *et al.*, 2012).

Reports on BR-deficient or BR-insensitive mutants and transgenic plants with altered seed characteristics and seed yield are more numerous in rice. Knockout or down regulation of BR biosynthetic genes and positive regulators of the BR signaling pathway in rice, generally resulted (except in case of gene redundancy) in sterility or strongly reduced seed yield due to smaller and rounder seeds (Reuzeau *et al.,* 2012).

2.5.6 Pathogen Resistance

When applied exogenously, BRs induce resistance against a broad range of diseases, notably against fungal and bacterial pathogens in tobacco and in rice. In rice, treatment with Brassinolide protected the plant against rice blast and bacterial rice blight diseases, whereas in tobacco, it enhanced resistance against the bacterial pathogen *Pseudomonas syringae* and the fungal pathogen *Odium* species (Reuzeau *et al.,* 2012).

BRs have been shown to reduce the damages caused by pesticides by accelerating their catabolism, consequently reducing their residual levels in the plants. Hence, BRs may be promising molecules suitable for application on crops to diminish the risks and deleterious effects associated with the exposure of humans, crops, and the environment to agrochemical substances (Bajguz and Hayat, 2009)

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