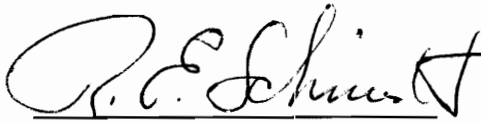


**Influence of Plant Growth Regulators on Turfgrass  
Polar Lipid Composition, Tolerance to Drought  
and Salinity Stresses, and Nutrient Efficiency**

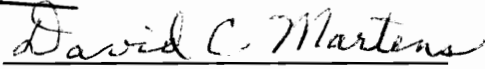
by  
JiYu Yan

Dissertation submitted to the Faculty of the  
Virginia Polytechnic Institute and State University  
in partial fulfillment of the requirements for the degree of  
Doctor of Philosophy  
in  
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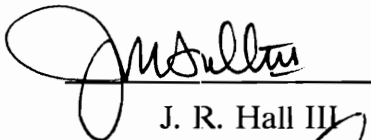
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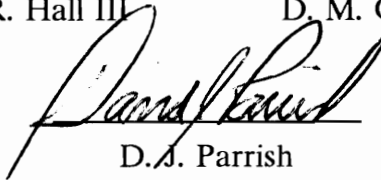
D. C. Martens, Co-Chairman



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D. J. Parrish

March, 1993  
Blacksburg, Virginia

**Influence of Plant Growth Regulators on Turfgrass  
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(ABSTRACT)

Two groups of plant growth regulators (PGRs), seaweed extract and triazole chemical, have been used in turfgrass management for improving turf quality and strengthening turfgrass tolerance to environmental stress. In order to understand the physiological functions and stress-tolerance mechanisms of the PGRs on turfgrass, a series of studies were conducted with perennial ryegrass (*Lolium perenne* L.). Ryegrass was treated with or without propiconazole (PPC) (1-((2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl)methyl)1H-1,2,4-triazole) and a proprietary fortified seaweed extract (FSE), which were combined with or without drought and salinity stress treatment. Total lipids, fatty acid composition of polar lipids, and total free and conjugated sterols were determined by the thin layer chromatograph, gas chromatograph, and scanner densitometer. This study indicates that PPC and FSE affected the unsaturation of polar lipid fatty acids and concentration of free sterols, which are major factors in changing cell membrane fluidity and permeability. The PGR-induced alteration of cell membrane lipid composition could be an adaptive

process to protect plant membrane function under drought and salinity stresses.

However, the metabolic effects of PPC and FSE may be different. It was found that PPC had a strong influence on unsaturation of polar lipid fatty acids, whereas FSE had a strong effect on free sterol concentration. Furthermore, a radish cotyledon expansion bioassay analysis showed that the FSE had cytokinin or cytokinin-like activity and could stimulate endogenous cytokinins in ryegrass, whereas an inhibition of cell expansion was seen in PPC-treated plants.

The possibility of using the PGRs to reduce fertilizer requirements was also studied. A higher uptake efficiency of most essential elements was found in PPC- and FSE-treated Kentucky bluegrass (*Poa pratensis* L.) than in the control (without PGR treatments). This effect was greater at lower than at higher fertilization levels. The utilization efficiency of some major nutrient elements also was higher in PPC- and FSE-treated bluegrass than in the control. The possibility of reducing fertilization by PGR application is positive.

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# Table of Contents

<b>Chapter 1. Introduction and Objectives</b> .....	1
<b>Chapter 2. Literature Review</b> .....	2
Plant Hormones and Plant Growth Regulators .....	2
Two PGRs: Triazole Chemical and Seaweed Extract .....	5
Propiconazole .....	6
Seaweed Extract .....	8
Cell Membrane Function and Composition .....	12
Cell Membrane Fluidity .....	13
Membrane Homeostatic Response .....	14
Effects of Environmental Factors on Membrane Composition .....	15
Salinity Stress and Membranes .....	15
Drought Stress and Membranes .....	16
Other Environmental Factors and Membranes .....	17
Growth Substances and Membrane Permeability .....	18
Cytokinin .....	18
Gibberellic Acid, Auxin, and Abscisic Acid .....	19
Environmental Stress and Growth Substance Responses .....	20
Drought Resistance and Growth Substances .....	21

Salinity Resistance and Growth Substances .....	22
References .....	25

**Chapter 3. Bioassay of Cytokinin Activity in Plant Growth Regulators and Growth**

<b>Regulator-Treated Ryegrass .....</b>	<b>41</b>
Introduction .....	41
Materials and Methods .....	42
Radish Cotyledon Expansion Bioassay for PGRs .....	43
Radish Cotyledon Expansion Bioassay of Endogenous Cytokinin Extracted from PGR-Treated Ryegrass .....	45
Results and Discussion .....	45
References .....	56

**Chapter 4. Alteration of Ryegrass Polar Lipid Composition Related to Drought**

<b>Stress and Propiconazole .....</b>	<b>58</b>
Introduction .....	58
Materials and Methods .....	60
Total Lipid Extraction .....	61
Polar Lipid Extraction and Fatty Acid Analysis .....	62
Sterol Identification and Quantification .....	63
Results .....	64
Discussion .....	70
References .....	74

<b>Chapter 5. Effects of Fortified Seaweed Extract on Polar Lipid Composition and Drought Tolerance in Ryegrass</b> .....	77
Introduction .....	77
Materials and Methods .....	79
Results .....	80
Discussion .....	87
References .....	91
<b>Chapter 6. Effects of Plant Growth Regulators on Polar Lipid Composition and Salinity Resistance</b> .....	96
Introduction .....	96
Materials and Methods .....	98
Results .....	99
Discussion .....	107
References .....	113
<b>Chapter 7. The Influence of Plant Growth Regulators on Turf Quality and Nutrient Element Efficiency</b> .....	117
Introduction .....	117
Materials and Methods .....	119
Results and Discussion .....	120
References .....	131

**Chapter 8. Summary** ..... 134

**Vita** ..... 138

# List of Illustrations

Figure 3.1. Standard curve showing the linear relationship between logarithmic value of 6-benzylaminopurine (BA) concentration ( $\mu\text{M}$ ) and fresh weight of radish cotyledons (mg) . . . . . 49

Figure 3.2. Standard curve showing relationship between 6-benzylaminopurine (BA) concentration ( $\mu\text{M}$ ) and fresh weight of radish cotyledon (mg) . . . 50

Figure 3.3. Relationship between concentration of fortified seaweed extract (FSE) ( $\mu\text{l ml}^{-1}$ ) and fresh weight of radish cotyledons (mg) . . . . . 51

Figure 3.4. Relationship between concentration of concentrated seaweed extract (CSE) ( $\mu\text{l ml}^{-1}$ ) and fresh weight of radish cotyledons (mg) . . . . . 52

Figure 3.5. Relationship between concentration of propiconazole (PPC) ( $\mu\text{l ml}^{-1}$ ) and fresh weight of radish cotyledons (mg) . . . . . 53

Figure 3.6. Relationship between concentration of triadimefon (TRF) ( $\text{mg ml}^{-1}$ ) and fresh weight of radish cotyledons (mg) . . . . . 54

## List of Tables

Table 3.1. The fresh weight (mg) of radish cotyledons grown in the endogenous cytokinin solution extracted from perennial ryegrass treated with plant growth regulators (PGRs) . . . . .	55
Table 4.1. Total lipids and total polar lipid fatty acids in leaves of perennial ryegrass treated with or without propiconazole and drought stress . . . . .	66
Table 4.2. Polar lipid fatty acid concentration in leaves of perennial ryegrass treated with or without propiconazole and drought stress . . . . .	67
Table 4.3. Double bond index, leaf water potential, and ratio of free sterols to polar lipids in leaves of perennial ryegrass treated with or without propiconazole and drought stress . . . . .	68
Table 4.4. Free sterols and conjugated sterols in leaves of perennial ryegrass treated with or without propiconazole and drought stress . . . . .	69
Table 5.1. Total lipids and total polar lipid fatty acids in leaves of perennial ryegrass treated with or without fortified seaweed extract and drought stress . . . . .	83
Table 5.2. Polar lipid fatty acid concentration in leaves of perennial ryegrass treated with or without fortified seaweed extract and drought stress . . . . .	84
Table 5.3. Double bond index, ratio of free sterols to polar lipids and leaf water potential in leaves of perennial ryegrass treated with or without fortified seaweed extract and drought stress . . . . .	85
Table 5.4. Free sterols and conjugated sterols in leaves of perennial ryegrass treated	

with or without fortified seaweed extract and drought stress . . . . .	86
Table 6.1. Double bond index, ratio of free sterols to polar lipids and leaf water potential in leaves of perennial ryegrass treated with or without propiconazole, fortified seaweed extract, and salt (NaCl) . . . . .	102
Table 6.2. Total lipids and total polar lipid fatty acids concentration in leaves of perennial ryegrass treated with or without propiconazole, fortified seaweed extract, and salt (NaCl) . . . . .	103
Table 6.3. Polar lipid fatty acid concentration in leaves of perennial ryegrass treated with or without propiconazole, fortified seaweed extract, and salt (NaCl) . . . . .	104
Table 6.4. Free sterols and conjugated sterols in leaves of perennial ryegrass treated with or without propiconazole, fortified seaweed extract, and salt (NaCl) . . . . .	105
Table 6.5. Concentration of K, Ca, Na, and Cl in leaves of perennial ryegrass treated with or without propiconazole, fortified seaweed extract, and salt (NaCl) . . . . .	106
Table 7.1. Density of Kentucky bluegrass treated with different fertility and plant growth regulators (PGRs) . . . . .	122
Table 7.2. Color rating of Kentucky bluegrass treated with different fertility and plant growth regulators (PGRs) . . . . .	123
Table 7.3. Clipping yield (gm <sup>-2</sup> ) of Kentucky bluegrass treated with different fertility and plant growth regulators (PGRs) . . . . .	124
Table 7.4. Internal concentration of nutrient elements in Kentucky bluegrass treated	

with different fertility and plant growth regulators (PGRs) . . . . .	125
Table 7.5. Nutrient element uptake efficiency in Kentucky bluegrass treated with different fertility and plant growth regulators (PGRs) . . . . .	127
Table 7.6. Nutrient element utilization efficiency in Kentucky bluegrass treated with different fertility and plant growth regulators (PGRs) . . . . .	130

# **Chapter 1**

## **Introduction and Objectives**

Extensive interest has been developed in utilizing plant growth regulators (PGRs) in turfgrass management during the past two decades. Most turfgrass PGR studies have been concerned with PGR effects on growth reduction to reduce mowing frequency or the PGR function as a herbicide. Research results indicate that proper use of certain PGRs may improve turf quality and benefit the environment. Recent research has focused on the PGRs with multiple functions. The PGRs could not only regulate plant growth and development as desired, but also increase plant resistance to disease, insects, and to environmental stress, as well as reduce plant requirement for fertilizer. Application of selected PGRs could reduce irrigation, fertilization, and pesticides requirement, and therefore reduce the risk of ground water pollution without sacrificing turf quality.

Seaweed extract (SE) and triazole chemicals are two different PGRs. There are numerous studies which prove that SE has cytokinin-like and auxin-like properties and can stimulate endogenous cytokinin activity (Crouch, 1990). Triazole chemicals inhibit gibberellic acid biosynthesis (Flecher, 1985). When applied to turfgrass, they enhance root growth, maintain green color, delay senescence (Schmidt, 1990; Goatley,

1988), and improve tolerance to drought and salinity stresses (Nabati, 1991). However, the physiological mechanism of these PGRs is not clear. Although these PGRs directly or indirectly have impact on plant growth substances which in turn affect the stress tolerance, nutrient uptake and utilization efficiency, little is known about the metabolism involved.

During recent decades, plant cell membranes have been recognized as a sensitive site for response to environmental changes in plant cells (Heber and Santarius, 1973). The changes in membrane fluidity can directly affect the activity of enzymes, permeability of cell solutes, and therefore change physiological and biochemical reactions. The major components of cell membranes, phospholipids and free sterols, are important in adjusting membrane fluidity (Shinitzky, 1984). Whether plants tolerate or resist environmental stress depends on the responses of plant cell membranes. Usually more-tolerant species have more efficient capability to adjust their membrane fluidity to an optimal state when they are exposed to stresses (Shinitzky, 1984). Many drought or salinity-sensitive cultivars have different patterns of alteration of membrane phospholipid unsaturation and free sterol concentration from the drought or salinity-tolerant cultivars when subjected to either drought or salinity stress (Kamell and Losel, 1989; Pham Thi, 1989; Erdei et al., 1980). However, it is unknown whether SE and triazole chemicals affect stress tolerance via the influence on cell membrane fluidity, or whether it is possible to reduce fertilization by use of PGRs. Information on these relationships is important to understand the function of the PGRs, to efficiently manage turfgrass, and to help find new PGRs.

Thus the objectives of this study are as follows:

- (i) to evaluate the cytokinin-like characteristics of the SE and triazole chemicals by a bioassay procedure.
- (ii) to determine the influence of a fortified seaweed extract (FSE) and a triazole chemical propiconazole (PPC) on cell membrane composition under the well-watered and drought stress conditions.
- (iii) to determine the influence of FSE and PPC on cell membrane composition under normal and salt stress conditions.
- (iv) to investigate the possibility of using the FSE and PPC to enhance nutrient efficiency and reduce fertilization requirements.

## **Chapter 2**

### **Literature Review**

#### **Plant Hormones and Plant Growth Regulators**

Plant hormones are naturally-occurring plant substances which were active in extremely small amounts, controlling (presumably via concentration) or influencing (via changes in cell sensitivity to the growth substance) various plant processes, such as growth and development. They fall into five classes: auxins, cytokinins, gibberellins, ethylenes, and abscisic acids (Salisbury and Marinos, 1985).

However, plant growth regulators (PGR), as defined by Nickell (1982), are organic compounds, other than nutrients, that affect the physiological processes of growth and development in plants when applied in low concentration. They can be either natural or synthetic compounds that are applied directly to a target plant to alter its life process or its structure, to improve quality, to increase yield, or to facilitate harvesting. The PGR is a broad concept, it is not restricted to synthetic components, but also includes the natural plant hormones. The term "plant growth substances" has same meaning as the PGR, specially implying the substances with the characteristics of plant hormones.

Generally, application of exogenous PGRs could produce their effects through

changing the endogenous plant hormones quantitatively and qualitatively, and allowing a modification of growth and development in the intended direction.

Recently more attention has been given to PGRs with multiple functions. They not only regulate plant growth and development as desired, but also increase the resistance to diseases and insects and enhance tolerance to environmental stress, such as drought, salinity, or temperature stress.

In recent years, the application of PGRs in agriculture and turfgrass management has become widespread. Although breeders continue to breed superior genotypes, and to generate many new varieties each year (Chalmers, 1990), PGRs are still an important tool for improving turf quality, such as turf color, density, resistance to fungi, insects and environmental stresses.

Some PGR may affect plants by modifying cell membranes (a rapid growth response) or by acting on enzymes formed after application (a delayed growth response) (Nickell, 1982). However, the effects of PGRs on plants may vary according to plant age, organs, physiological status, nutritional status, and environmental conditions.

## **Two PGRs: Triazole Chemical and Seaweed Extract**

Two types of PGRs were used in this study. One is a synthetic material, triazole chemical, which functions both as a fungicide and as a PGR. The other is a natural product extracted from seaweeds.

## **Propiconazole**

Propiconazole (PPC) is a triazole fungicide used on turfgrass for controlling dollar spot (*Sclerotinia homoencarpa*), red thread (*Corticium fueiforme*), rust (*Puccinia graminis*), powdery mildew (*Erysiphe graminis*), stripe smut (*Ustilago striiformis* and *Urocystis agropyri*), summer patch (*Magnaporthe poae*), spring dead spot (*Pyricularia grisea*), pink snowmold (*Fusarium nivale*) and gray snowmold (*Typhula* spp.) (Ciba-Geigy, 1990). Like other triazole fungicides, PPC inhibits fungal sterol biosynthesis. All known triazole fungicides are demethylation inhibitors which have an sp<sup>2</sup>-nitrogen atom with a free electron pair. This free electron pair can bind to the center of the ferric porphyrin system of cytochrome P-450 and prevent the formation of the first oxygenated complex (Vanden Bossche, 1985; Vanden Bossche et al., 1985; 1986a; 1986b; Yoshida and Aoyama, 1986). Specifically, the triazole fungicides inhibit C-14 demethylation of 24-methylenedihydrolanosterol by binding cytochrome P-450 mixed-function oxygenase, and therefore inhibiting the biosynthesis of ergosterols (Kato, 1986; Sisler and Ragsdale, 1984). The PPC inhibits fungal growth and reproduction, since the biosynthesis of ergosterol is required for reproduction and membrane function (Koller, 1987).

A significant side effect of triazole fungicides on free fatty acid composition of fungi has been reported (Sisler and Ragsdale, 1984; Vanden Bossche, 1985; Weete, 1987). Free fatty acids increased in concentration and shifted to a higher degree of unsaturation, which may represent an adaptive response of the fungi to changes in membrane sterol composition. The membrane sterol and fatty acid composition can

be regulated directly by triazole fungicides (Low et al., 1985).

The demethylation of sterols at the 14-C position is not restricted to fungi. It also occurs in some higher plants (Benveniste, 1986; Rahier and Taton, 1986). Most plants treated with triazole fungicides exhibit "side effects", such as reduced shoot and leaf growth, reduced transpiration and increased yield under water stress conditions, enhanced protection from injuries caused by chilling or heat (Fletcher and Hofstra, 1985; 1988; Davis et al., 1988; Fletcher, 1985), improved anti-senescence properties, enlarged chloroplast (Gao et al., 1988), enhanced chlorophyll content (Fletcher and et al., 1986; Izumi et al., 1988), and decreased gibberellin concentration (Davis et al., 1988; Fletcher and Hofstra, 1988). These "side effects" are viewed as beneficial rather than phytotoxic, so that these fungicides are considered to be plant growth regulators.

Some of the triazole chemicals are strong fungicides, while others are effective plant growth regulators. Some have both properties, depending upon the mixture of triazole isomers (Koller, 1987). Propiconazole is one having both functions.

Triazoles also inhibit gibberellin biosynthesis in plants (Sisler et al., 1984; Rademacher et al., 1984; Koller, 1987; Buchenauer and Rohner, 1981). The site of inhibition is the oxidation of *ent*-kaurene to *ent*-kaurenoic acid which is a precursor of gibberellin. The oxidation reaction, like sterol demethylation, is catalyzed by the cytochrome P-450 mixed-function oxygenase (Amrhein, 1983). The gibberellins are important growth substances involving in many physiological activities such as cell elongation and expansion, cell differentiation and synthesis of cell membrane phospholipids (e.g. phosphorycholine glyceride transferase, phosphorylcholine

cytidyltransferase) (Brock and Kaufman, 1991). The inhibition of gibberellin biosynthesis causes growth retardance and the other phenomena described previously.

The triazole PGRs are still in the development stage (Rademacher, et al., 1984). The influence of triazole chemicals on higher plant metabolism is less well understood than their effects on fungi. The question has been raised as to whether the triazoles have other functions regulating plant growth and development besides inhibiting GA biosynthesis.

### **Seaweed Extract**

Seaweed extract (SE) and seaweeds have been utilized in agriculture for centuries (Crouch, 1990), and are still attracting more attention.

Seaweed extract contains almost all the major and minor nutrient elements (Stephenson, 1968), at least 17 common amino acids (Munda and Gubensek, 1975), and vitamins B1, B2, C, E (Hundin and Ericson, 1956; Teeri and Beiber, 1958). Antibacterial, antifungal (Screenivasa Rao and Parekh, 1981; Pesando and Caram, 1984; Okami, 1982), and antiviral activities (Ehressmann, et al., 1977) were also found in some seaweeds and SE. It is well documented that SE contains cytokinins, auxins, GAs, and ABA-like growth substances. Cytokinin or cytokinin-like components were identified in all commercial SE and seaweed (Bently-Mowat and Reid, 1968; Hussian and Boney, 1969; Mooney and Van Staden, 1986; Brain et al., 1973; Blunden and Wildgoose, 1977; Finnie and Van Staden, 1985). Auxin-like substances (Williams, et al., 1976; Sanderson and Jameson, 1986; Kingman and Moore, 1982; Sumera and Cajipe, 1981), gibberellin-like substances (Mowat, 1965; Jennings, 1968; Taylor and

Wilkinson, 1977; Wildgoose, et al., 1978), and Abscisic acid-like substances (Hussian and Boney, 1973; Kingman and Moore, 1982) were also found in SE, but their activity was regarded as lower than that of cytokinin.

The benefits of SE for plants are well-documented. Increased plant shoot growth, branching, and tuberization by SE treatment have been reported in many species (Featonby-Smith and Van Staden, 1983a; Aldworth and van Staden, 1987; Senn and Skelton, 1969; Temple and Bomke, 1989; Nelson and Van Staden, 1986; Crouch, 1990).

Increased root length, fresh weight (Blunden and Wildgoose, 1977; Blunden et al., 1981; Featonby-Smith and Van Staden, 1983b), and improved lateral root development (Finnie and Van Staden, 1985; Metting et al., 1990) had been found in SE treated plants.

Francki (1960a, 1960b) found higher  $Mg^{++}$  concentration in SE-treated tomato leaves. Lynn (1972) showed evidence of improved uptake of B, Cu, Fe, Mg, and Zn in SE treated green peppers. Enhanced uptake of Ca, K, and Mg in lettuces (Crouch et al., 1990) and increased availability of soil N, P, K, Ca, Mg, Mn, Fe, and Zn in wheat and tomato have been reported (Caiozzi et al., 1968; Beckett and Van Staden, 1989, 1990a, 1990b; De Villiers et al., 1983).

Enhanced resistance to fungi, bacterial and insect attack has been observed in a variety of plants treated with SE (Senn et al., 1961; Stephenson, 1966; Hall, 1975). Reduced incidence of nematode infection also has been reported in SE treated plants (Stephenson, 1968; Featonby-Smith and Van Staden, 1983).

The application of SE on plants has been shown to increase fruit and seed yield

(Aitken and Senn, 1965; Blunden, 1972; Nelson and Van Staden, 1986), to delay senescence and ripening of fruit (Driggers and Marucci, 1964; Skelton and Senn, 1969; Blunden et al., 1978), to break seed dormancy, and to increase germination (Wilczek and NG, 1982; Button and Noyes, 1964).

Although several explanations have been proposed, the mechanism of SE actions is uncertain. Initially, seaweed was used as meal and blended with the soil in large volumes. The mineral elements in the seaweed were considered to be fertilizer to supplement nutrients for plant growth (Stephenson, 1974; Senn and Kingman, 1978). The alginic acids such as D-mannuronic and L-guluronic acid residues, which comprise about one-third of total carbohydrate content in SE, have chelating ability with some trace minerals in soil to yield soluble complexes and increase availability for plants. Seaweed meals also were used as soil conditioners, since they improve aeration and aggregate stability (Quastel and Webley, 1947). Improved soil structure and supplemental nutrition increased by seaweed meal generated more suitable conditions for root growth. The better developed root system would enhance nutrient and water uptake, so that whole plant growth, vigor, and resistance were improved.

Later, seaweed products were used as liquid extracts applied to foliage. The optimum concentration for foliage application is very low, the mineral elements contained would be too low to assume any significant benefit on plant growth (Abetz, 1980). Finnie and Van Staden (1985) found that ash SE lost its stimulatory effect in plants. This implies that the regulatory effect of SE is associated with some organic components rather than minerals (Crouch, 1990).

Recently, the endogenous growth substances in SE have been considered as the

major active components related to the beneficial effects. Furthermore, many experiments showed that results obtained from the use of SE were similar to those obtained from the use of cytokinin (Crouch, 1990; Schmidt, 1990).

Cytokinins or cytokinin-like substances may be the principal active components in SE. Most of the research supports this view. The physiological responses of plants to cytokinin are improved protein synthesis and cell division, enhanced nutrient mobilization, delayed senescence, increased lateral bud growth, and inhibited fungal infection (Brock and Kaufman, 1991). However it is hard to confirm that cytokinin or cytokinin-like substances alone are the sole active components, since not all the benefits from SE could be explained by cytokinin. Other growth substances, like GA, auxin, or ABA, and/or their interaction with cytokinin may also contribute to the observed responses previously discussed.

#### **Fortified Seaweed Extract (FSE):**

Most commercial SE are concentrated or fortified. The SE product used in this study is fortified by adding humic acid extracted from peat moss, vitamin B (Thiamin), and intermediate metabolites (L-ascorbic acid).

The effect of humic acid (HA) on plant growth has been discussed for nearly 50 years. Humic acids could improve nutrient uptake (Jelenic et al., 1966) and stimulate total root growth (Bystricka and Sladky, 1973). However, the specific regulatory mechanism of HA on plant metabolism is not certain. There are several hypotheses which explain the effects of HA at different levels, such as: the formation of complexes between HA and mineral ions (Flaig, 1966), catalysis of HA to different

enzymes in the plant (Kononova, 1966), influence of HA on respiration and photosynthesis (Hernando, 1968), stimulation of nucleic acid metabolism and of mitotic activity of the meristem (Ali-Zade and Gadzhieva, 1977; Aliev et al., 1984).

## **Cell Membrane Function and Composition**

Cell membranes serve not only as selective permeability barriers for compartmentalization, but also serve as the structural matrix for proteins carrying enzymes, carriers, proton pumps, and energy regulating components control the active transport processes (Devlin and Witham, 1983). Lehninger (1975) stated that the most satisfactory model of membrane structure is the fluid-mosaic model, proposed by S. J. Singer and G. L. Nicolson in 1972. This model postulates that membranes consist of a fluid phospholipid bilayer with globular protein molecules penetrating into either side or extending through the membrane. Free sterols are inserted into membrane bilayer. Individual lipid molecules and proteins are free to diffuse laterally in two dimensions, which endows the bilayer with fluidity, flexibility, and selective permeability. Polar lipids are mostly localized in the membranes. Among the polar lipids, the phospholipids are predominate lipids (Lehninger, 1975).

Lipids are important determinants and regulators of membrane function. Subtle changes in lipid structure and composition can dramatically alter membrane properties which can be physiologically important to the cell (Lynch and Thompson Jr., 1988).

## Cell Membrane Fluidity

Membrane fluidity is a physicochemical concept. It reflects the intrinsic characteristics of lipids present in the membrane, such as lipid nature, arrangement, composition, and the interaction of individual molecules (Crowe and Crowe, 1988). These physico-chemical properties directly influence membrane function biologically and physiologically (McMurchie, 1988).

The phospholipid fatty acyl chains within the hydrocarbon core of membrane bilayers are major contributors to membrane fluidity. In general, a higher degree of unsaturation and longer fatty acyl chains of polar lipids increase membrane fluidity. The saturated fatty acids, mainly  $C_{16:0}$  and  $C_{18:0}$ , tend to decrease membrane fluidity (Quinn, 1981; Subbs, 1983; Subbs and Smith, 1984). The asymmetric distribution of phospholipids and fatty acyl chains could result in differences in lipid fluidity in each half of the bilayer (Wisnieski and Iwata, 1977; Schroeder, 1980).

Free sterols in membranes form a complex with the membrane phospholipids both by hydrophobic interactions and by hydrogen bonding (Franks, 1976; Yeagle, 1985). The free sterol level or the ratio of free sterol to phospholipids could dramatically influence membrane fluidity (Cooper and Strauss, 1984), permeability (Demel et al., 1972), and enzyme and receptor functions (Demel and De Kruijff, 1976). An increase in the free sterol content significantly decreases fatty acyl chain mobility and induces an intermediate state of fluidity (Stockton and Smith, 1976, Van Dijck et al., 1976). As Leshem (1992) explained: "Since the steroid skeleton is rigidly planar, sterols on the one hand cause membranes to be more rigid or, in other words, they increase microviscosity. On the other hand, the hydrophobic and aliphatic sterol

tail which inherently is mobile introduces a certain degree of disorder in the microdomain of the membrane with which it is associated -- it thus has an opposite and fluidizing effect. Taken together the above findings indicate that membrane sterols have a homeostatic and stabilizing effect which enable cellular function over a wide temperature range including diurnal and ambient temperature which may fluctuate to the extent of 30-40 °C ".

Sterols exist in the free and conjugated formes. The conjugated sterols, such as steryl glycosides (SG), steryl esters (STE), and esterified steryl glycosides (ESG), may serve translocation and storage functions. Interconversion between free and conjugated sterols could maintain a certain level of sterol in the membrane to serve the optimum membrane function (Cooper and Strauss, 1984; Yeagle, 1985).

Differences of phospholipid head groups in size, shape, polarity, and charge could influence membrane fluidity, permeability, and other properties (Boggs, 1986). The particular proteins and the ratio of protein to lipid in membrane are important factors in influencing membrane fluidity (Di Costanzo et al., 1983).

## **Membrane Homeostatic Response**

Optimum membrane fluidity is necessary for the normal function of cells, because many membrane-associated functions involving enzymes, receptors, and ion channels are modulated by changes in membrane fluidity (Sandermann, 1978; Quinn, 1981; Subbs and Smith, 1984). When a plant is subjected to stress, the membrane generally is modified for restoration of membrane fluidity to an optimal state (Cornan and

Gelmann, 1975; Hazel and Prosser, 1974). This process is a homeostatic response, mainly proceeding via alteration of the degree of unsaturation of phospholipid fatty acids (Sinensky, 1974) and/or changes in the level of membrane sterols (Chin et al., 1978). This process is an important mechanism for maintaining optimum membrane fluidity and protecting membrane function under changing growth conditions. Poor homeostatic capability of plant cell membrane could result in poor adaption to changing environmental conditions.

## **Effects of Environmental Factors on Membrane Composition**

### **Salinity Stress and Membranes**

Polar lipids and free sterols change quantitatively and qualitatively under salinity stress. Erdei et al. (1980) found that phospholipid levels in the roots of salt-tolerant *Plantago* species were lower than that in salt-sensitive *Plantago* species which grown under non salt condition, while the phospholipid level increased in the salt-tolerant species, and decreased in the salt-sensitive species under salinity stress. Similar changes were found in barley (*Hordeum Vulgare* L.) (Ferguson, 1966). Increased unsaturation degree of membrane lipids was found to directly enhance K<sup>+</sup> permeability and reduce the accumulation of Na<sup>+</sup> (Scarpa and de Gier, 1971), which reduced ionic toxicity. Also it has been shown that salt-tolerant species contain a higher level of C<sub>18:2</sub> and lower level of C<sub>18:3</sub> than the salt-sensitive species (Stuiver et al., 1978). Reduced unsaturation in fatty acid composition might lessen the fluidity of the membrane and decrease its permeability to ions, and could be part of the

regulatory process of salt resistance in plants (Harzallah-Skhiri et al., 1982). Curtain (1988) also indicated that high NaCl could lead to rigidification of cell membranes. The rigidification could influence the activity of membrane-bound enzymes by reducing effective surface charge, and increase glycerol production (Fontana and Haug, 1982; Craigie and McLachlan, 1964). The increase of glycerol concentration could eliminate the requirement and uptake of NaCl (Curtain, 1988).

Free sterols are an effective factor in the regulation of membrane stability and in the reduction of passive ionic permeability. In grape varieties differing in salt resistance, the lowest free sterol level was found in the most sensitive variety (Kuiper, 1968). The ratio of sterol to phospholipid was higher in salt-resistant *Debaryomyces hansenii* than the salt-sensitive *Sacharomyces cerevisiae* (Adler and Liljenberg, 1981). The level of sterol esters increased in sugar beet (Stuiver et al., 1981) and in a halophytic species (Erdei et al., 1980) when these plants were exposed to salinity.

### **Drought Stress and Membranes**

Membrane lipids can be modified in both concentration and composition by changes in the availability of water. Generally, drought stress results in a decrease in polar lipid components and an increase in neutral lipids (Parks et al., 1984; Navari-Izzo et al., 1989). Phospholipid content was lower in a drought-resistant variety than that in a drought-sensitive variety of wheat (*Triticum turgidum* L. var. durum); however, after drought stress treatment, total phospholipids concentration increased in the drought-resistant variety and decreased in the drought-sensitive variety (Kamell and Losel, 1989). A similar change was found in cotton leaves between a drought-

resistant variety and a drought-sensitive variety (Pham Thi, 1989).

A decrease in membrane lipid concentration might be caused by a reduction in the total membrane area of the cell (Norberg and Liljenberg, 1991), or temporary shrinkage of the membrane area as hydrostatic pressure is reduced.

Whether free sterols increase or decrease in concentration depends on the duration and severity of water stress (Douglas and Paleg, 1981; Simonds and Orcutt, 1988). Liljenberg et al. (1985) found that free sterol content was slightly decreased after two periods of water-deficit stress, and increased after four stress periods in oat (*Avena sativa* L.) root cells. Several inductive cycles or a critical degree of water stress were required for certain modifications of sterol metabolism. Sterol synthesis might also be mediated via the abscisic acid-cytokinin balance of cells in oat seedlings (Liljenberg et al., 1985).

### **Other Environmental Factors and Membranes**

Generally, the degree of unsaturation of phospholipid fatty acids increase at reduced temperatures (Fukushima et al., 1976). The chill-resistant alga (*Anacystis nidulans*) showed increased lipid unsaturation when grown at lower temperatures (Kuiper, 1985). Lipid unsaturation seems to be an active factor in temperature adaptation. At a lower temperature, the advantages of increased membrane fluidity or increased lipid unsaturation are that membranes can remain functioning more easily, and proteins can retain their native configuration and thus activity.

Deficiency of molecular oxygen (anaerobic stress) could lead to a decrease in lipid formation and unsaturation of long-chain fatty acids, and cause the membrane

less fluid in yeast (Nozawa and Vmeki, 1988).

Alteration of pH could change charge distribution at the polar headgroup, thus being able to induce a lipid fluidity (Jacobson and Papahadjopoulos, 1975).

## **Growth Substances and Membrane Permeability**

### **Cytokinin**

Plant growth substances play a critical role in plant growth, development, and acclimation to environmental stress. Veldstra (1944), and many others since, have suggested that one of the principal functions of growth substances is their effect on membrane permeability.

Feng and Unger (1972) and Feng (1973) reported that permeability of kinetin-treated onion (*Allium cepa* L.) epidermal cell membrane was increased to glycerol, urea, thiourea, and other nonelectrolytes. Also kinetin treatment could significantly increase permeability to more polar molecules (i.e. urea thiourea) than to less polar components (i.e. glycerol). This suggests that kinetin treatment changes the selective permeability via changes of protein phase of the membrane system. Kinetin can directly affect RNA synthesis and consequently protein synthesis (Letham, 1967; Brock and Kaufman, 1991).

However, in many protein-free lipid bilayer systems, kinetin still can increase permeability. Stillwell and Hester (1983) found that kinetin can increase the water permeability of either natural or artificial lipid bilayers. Furthermore, not only water which is a small neutral molecule, but also glucose which is a larger molecule were

rendered more permeable through the lipid bilayer under kinetin treatment (Bangham et al., 1967; Wood and Wirth, 1968). However, permeability enhancement by kinetin was only detected in the liquid-crystal state of membrane, but not in the gel state. Isomers of zeatin were found to influence bilayer permeability differently. The *trans*-isomer of zeatin was ineffective in enhancing permeability, while *cis*-zeatin was very effective in enhancing permeability (Stillwell et al., 1985). Therefore, kinetin can interact with certain components of lipid bilayers and change membrane fluidity and permeability. However, the alteration of membrane lipids might be related to their protein components.

### **Gibberellic Acid, Auxin, and Abscisic Acid**

Gibberellic acid was found to increase the permeability of glucose, sucrose, and ions in membrane model systems (Wood and Paleg, 1972). The increase in permeability seemed relate to biophysical alteration in lipid composition (Wood and Paleg, 1972).

Auxins have been shown to interact with phosphatidylcholine (Marker et al., 1978). Glinka (1971) reported that abscisic acid could increase membrane permeability to water in carrot cylinders.

In order to better understand the relative effects of these different growth substances on membrane permeability, Hester and Stillwell (1984) tested the four groups of plant growth substances (auxins, GAs, ABAs, and cytokinins) with respect to their influence on the permeability of erythritol and urea. The results indicated that all four groups of growth substances can stimulate an increase in membrane

permeability as their concentration was increased. However, cytokinins (kinetin and benzyladenine) were most effective at enhancing bilayer membrane permeability, while auxins, GA, and ABAs affected permeability to urea and erythritol only at very high concentrations, ranging from 1300  $\mu\text{M}$  (for auxins and ABAs) to 13000  $\mu\text{M}$  (for GAs). Only 30  $\mu\text{M}$  of cytokinins was needed for similar permeability changes compared to other growth substances. It is possible that cytokinins more directly affect membrane permeability, while auxins, GAs, and ABAs could affect membranes in some unknown manner.

## **Environmental Stress and Growth Substance Responses**

Plants live in a constantly fluctuating environment. Some environmental changes are small and occur periodically (such as day length, season, etc.), and plants have adapted to these changes. Other environmental changes are less regular and more damaging to plants (such as drought, extreme temperature variation, salinity, etc.). Thus plants need to rapidly adapt to these changes (Reid et al., 1991).

Whether plants survive or die depends on their response to stress. Levitt (1980) precisely defines stress as the external factors acting on the organism. The effect of stress on a plant was defined as strain. Up to a point, which is specific for each plant, a strain may be completely reversible. Beyond this point, the strain may be partially reversible or irreversible. The reversible effect is called "elastic strain", and the irreversible effect is called "plastic strain". Plants are always attempting to change themselves physiologically and morphologically to become less stressed so as to avoid

plastic strain.

There are various mechanisms by which plants respond to environmental changes. Reid et al. (1991) has extensively reviewed these mechanisms. However, no matter what these mechanisms are, plant growth substances play a critical role. It is well documented that growth substances initially respond to environmental change, then react as long-distance messengers and amplifiers, sending the information through all parts of the plant. The plant can therefore protect itself from getting serious damage (Aspinal, 1980; Eder and Huber, 1977).

### **Drought Resistance and Growth Substances**

Drought stress is a common occurrence, since many factors could cause it, such as high or low temperature, high wind, low RH%, soil salinity, etc. However, plants have a certain capability to acclimate. When drought conditions appear, the ABA level in plants generally increases and cytokinin level decreases, causing stomata to close. This response allows plants to reduce further water loss (Schuize, 1986).

The ABA concentration was found highly related with plant water status (Reid et al., 1991). The plant root is the major site where ABA is produced (Robertson et al., 1985), and can act as sensors to changing soil water status (Zhang and Davies, 1989). Dehydrated roots can export ABA to leaves for changing stomatal conductance (Turner et al., 1985). However, Hartung et al. (1981) thought that leaf mesophyll cells are also locations for ABA biosynthesis and accumulation. The ABA may be made in mesophyll cytoplasm (Hartung, 1983) and move through the

apoplasts (Hartung et al., 1988) to the guard cells.

Cytokinins and GAs (Liven and Vaadia, 1965; Blackman and Davies, 1985) as well as IAA and ethylene (Levitt et al., 1987) could stimulate stomatal opening. When water is deficient, their concentration could decrease (Guinn and Brummett, 1988; Itai and Vaadia, 1965). The interaction between ABA and cytokinin has been viewed as a factor that allows the plant to adapt to stress. The ratio of ABA to cytokinin could be changed either as the ABA level rises and cytokinin level drops, or as the ABA level remains constant and cytokinin level drops. Both could cause stomatal closure (Itai and Benzioni, 1976; Mizrahi, 1980; Bradford, 1982). Blackman and Davies (1985) substantiated this hypothesis by using a split root technique on maize plants under mild water stress.

With longer and more extreme water deficit, some sugars, organic acids, and amino acids could accumulate (Singh et al., 1973; Hanson and Nelson, 1978; Cutler and Rains, 1978; Timpa et al., 1986). These accumulation decreases the osmotic potential and holds more water. Cytokinins are thought to be involved in these processes. It was found that cytokinin stimulates transportation of sugars and amino acids (Mothes and Engelbrecht, 1961), as well as  $^{32}\text{P}$ ,  $^{22}\text{Na}$ , and  $^{14}\text{C}$  to the cytokinin rich places (Muller and Leopold, 1966a, b; Gunning and Barkley, 1963). Cytokinin could catalyze the conversion of storage compounds to osmotically active compounds (Bewli and Witham, 1976).

Cytokinins can stimulate root growth and development which in turn increases water uptake and reduces drought stress (Reid et al., 1991).

## **Salinity Resistance and Growth Substances**

Saline soils are found commonly in such place as seacoasts, arid zones with high evaporation and under heavy irrigation (McWilliam, 1986). Excess salt in soil is harmful to plants since it decreases the availability of water, and because absorbed salt ions may become toxic.

Avoiding the uptake of excessive amounts of salt into a plant is an important way to resist saline stress for most non-halophytes. Some salt-resistant varieties take up less  $\text{Na}^+$  and  $\text{Cl}^-$  than the salt-sensitive varieties (Reid et al., 1991). This exclusion mechanism must depend on the cellular membrane properties, such as the decreased permeability to salt ions, and discrimination on selectivity to  $\text{Na}^+$  and  $\text{Cl}^-$ . Growth substances could also be involved in the membrane regulation. Singh et al. (1989) found that ABA treatment could induce a new mRNA and encode a new protein which only existed in salt-adapted tobacco cells. Exogenous ABA could stimulate osmotic adjustment through sugar accumulation (La Rosa et al., 1987) to overcome the saline stress.

Khan and Huang (1988) reported that added cytokinin, aminocyclopropane-1-carboxylic acid (ACC), and ethylene can overcome salt-induced inhibition of germination in lettuce seeds. It was assumed the exogenous cytokinin could stimulate endogenous ACC and ethylene which facilitated salt inhibition. Salt stress often results in physiological drought stress. So the regulatory response of growth substances to drought could be similar to the response induced with salt stress. The effect of other growth substances with regard to salt stress has been rarely investigated.

Functions of growth substances are very complicated in plant growth, development, and adaptation. One single growth substance could respond to many<sup>6</sup> different environmental changes, while a single environmental change could result in<sup>1/52</sup> variation in more than one growth substance. These phenomena are complicated and<sup>6</sup> difficult to appraise.

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## Chapter 3

# Bioassay of Cytokinin Activity in Plant Growth Regulators and Growth Regulator-Treated Ryegrass

### Introduction

Two groups of plant growth regulators (PGRs), seaweed extracts and triazole chemicals, have been reported to delay senescence, improve leaf color, enhance root growth, and decrease fungal infection when applied to turfgrass (Goatley and Schmidt, 1990; Goatley, 1988). These PGR-induced changes were consistent with the responses of plants to cytokinin applications (Brock and Kaufman, 1991).

It has been well documented that seaweed and SE contain cytokinin or cytokinin-like substances (Bently-Mowat and Reid, 1968; Hussian and Boney, 1969; Mooney and Van Staden, 1986; Brain et al., 1973). However, triazole chemicals have been considered as GA biosynthesis inhibitors since they can block the oxidation of *ent*-kaurene to *ent*-kaurenoic acid, which is a precursor of GA (Koller, 1987). There are no report concerning the interaction of triazole chemical and cytokinin.

Ascertaining the characteristics of the two seaweed extracts: fortified seaweed extract (FSE) and concentrated seaweed extract (CSE), and two triazole chemicals:

propiconazole (PPC) and triadimefon (TRF), and knowing whether these PGRs contain cytokinin or cytokinin-like substances are important to help explain their regulatory mechanism. The purposes of this study were to use a bioassay method to determine the presence of the cytokinin or cytokinin-like substances in the PGRs, to find the optimum active range in regulating grass growth, and to ascertain the influence of the PGRs on endogenous cytokinin level.

## **Materials and Methods**

### **Plant Growth Regulators**

Fortified seaweed extract (FSE) supplied by ROOTS Inc. (New Haven, CT). It was an extract from eight seaweeds: *Laminaria digitaria*, *Ascophyllum nodosum*, *Fucus vesiculosus*, *Sargassum sp.*, *Ulva sp.*, *Caulerpa sp.*, *Gelidium sp.*, and *Porphyra tenera*. The FSE was fortified by adding humic acid, vitamin C, and vitamin B<sub>1</sub>.

Concentrated seaweed extract (CSE) was supplied by Plant Growth Formulation Inc. (Gainesville, GA).

Propiconazole (PPC) contains 14.3% of active ingredient 1-((2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl)methyl)1H-1,2,4-triazole (Ciba-Geigy, Greensboro, NC).

Triadimefon (TRF) contains 25% of active ingredient 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazole-1-yl)-2 butanone (Miles Inc. Kansas City, MO).

6-Benzylaminopurine (BA) ( $C_{12}H_{11}N_5$ , mw=225.26) was supplied by Polaroid Corporation Chemical Commercial Development Department (Combridge, MA).

#### **Radish Cotyledon Expansion Bioassay for PGRs:**

One hundred grams of radish (*Raphanus satives*) seeds were soaked into a 5% sodium hypochlorite solution for 20 minutes, and then washed with distilled water. The washed seeds were germinated on paper saturated with distilled water in a tray. The tray was completely enclosed in a black polyethylene bag and incubated in a dark growth chamber at 25 °C for approximately 35 hours (Yopp et al., 1986).

After the radish seeds had germinated, the smaller of the two cotyledons of a selected number of seeds was removed from the hypocotyl. Seven uniform cotyledons were selected and transferred, upper surface down, to filter paper in Petri dishes. The filter paper was moistured with 3 ml of potassium phosphate buffer (2 mM, pH 6) as control, or contained the PGRs (FSE, CSE, PPC, and TRF) at a series of concentration showed in Fig. 3.3, 3.4, 3.5, and 3.6. Meanwhile, synthetic cytokinin, 6-benzylaminopurine (BA), was used to make a cytokinin standard curve. The Petri dishes, without covers, were then transferred to a plastic tray (50 cm X 70 cm) which had filter paper fully saturated with distilled water. The tray was covered with transparent polyethylene wrap and incubated at 25 °C under a continuous approximately  $1200 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$  of cool white fluorescent light in a growth chamber. After three days of incubation, the increase in weight gain over that of the control (buffer solution only ) was calculated. There were three replications for each PGR treatment, and the experiment was repeated three times.

### **Endogenous Cytokinin Extraction from PGR-Treated Ryegrass:**

**Culture of Perennial Ryegrass:** Perennial ryegrass (*Lolium perenne* L.) seeds 2.2 g were placed in 9 cm (diameter) Petri dishes and wetted with 5 ml of distilled water. The Petri dishes were then transferred into a large tray (50 cm X 70 cm) with a wet paper towel on the bottom. Then the tray was placed in a growth chamber with a 10-hour day (25 °C, 1200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and a 14-hour night (18 °C, darkness) photoperiod. Fifteen days later, the PGRs were applied to the ryegrass seedlings with a syringe. The concentration of the PGRs were 4  $\mu\text{l ml}^{-1}$  for FSE, 0.3  $\mu\text{l ml}^{-1}$  for CSE, 0.3  $\mu\text{l ml}^{-1}$  for PPC, and 3  $\text{mg ml}^{-1}$  for TRF respectively, and total 5 ml in each Petri dish. Each PGR treatment had five replications. Eight days after the PGR-treatment, the seedlings were harvested for extraction of endogenous cytokinin.

**Endogenous Cytokinin Extraction:** A total of 15 g of fresh leaf tissue was harvested from five Petri dishes of each PGR-treatment. Extraction of cytokinins from ryegrass leaf tissues was processed as Horgan (1978) described. The leaf tissue was chopped, frozen with liquid nitrogen, and ground with a mortar and pestle. Then 150 ml of methanol:chloroform:formic acid (v/v/v, 12:5:3) was used to extract the tissue, and was kept at -20 °C for 24 hours. Supernatant was obtained after centrifuging at 3300 rpm and -4°C. The residue was extracted with 75 ml of methanol:formic acid:water (v/v/v, 60:40:1) at -20 °C for 1 hour. The supernatant from the two extraction were combined and evaporated under reduced pressure. The extraction solution was evaporated to 1 ml and its pH value was adjusted to 6 with NaOH. This solution was partitioned 3 times with 5 ml of dethyl ether, and the

dethyl ether layers were combined. Potassium phosphate buffer solution 10 ml was added to the ether extract and the ether was evaporated under nitrogen gas. The residual aqueous containing the endogenous cytokinin extract was brought to 20 ml with the buffer solution for cytokinin bioassay.

**Radish Cotyledon Expansion Bioassay of Endogenous Cytokinin Extracted from PGR-treated Ryegrass:** Radish cotyledons were prepared as previously described. Two groups of incubation solutions were used to test cotyledon growth. The first group consisted of 4 ml of endogenous cytokinin extract from 3 g of fresh tissues in each of 3 Petri dishes. The second group consisted of a mixture of 2 ml of endogenous cytokinin extract from 1.5 g fresh tissues and 2 ml of buffer solution in each of 3 Petri dishes. Seven uniform radish cotyledons were grown in the incubation solution and weighed after 35 hours of incubation in growth chamber (25 °C, 1200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light). There were three replications for each different incubation solution treatment.

## **Results and Discussion**

### **Cytokinin Biological Activity of the PGRs:**

6-Benzylaminopurine (BA) was used as the standard cytokinin in this study. The logarithmic values of BA concentration showed a linear relationship with radish cotyledon fresh weight when BA concentration was in  $8.88 \times 10^{-2} \mu\text{M}$  to  $44.4 \mu\text{M}$  range (Fig. 3.1), which can be formulated as:

$$Y = 70.9 + 4.74 \ln(X) \quad (r^2 = 0.9514)$$

Where Y is a value of cotyledon weight (mg), and X is concentration of BA solution ( $\mu\text{M}$ ). The highest biological activity of BA in stimulating cotyledon cell expansion was at 44.4  $\mu\text{M}$ . Beyond this concentration, cotyledon weight did not significantly change with the increase of BA concentration (Fig. 3.2).

Cotyledon growth was stimulated with FSE concentrations up to 8  $\text{ul ml}^{-1}$  (Fig. 3.3). The optimum concentration was 3  $\text{ul ml}^{-1}$ , which was biologically equivalent to 2.26  $\mu\text{M}$  of BA. However, when the FSE concentration was higher than 10  $\text{ul ml}^{-1}$ , cotyledon growth was inhibited (Fig. 3.3).

Cotyledon growth was also stimulated by CSE. The active range of CSE from 0.1 to 0.4  $\text{ul ml}^{-1}$  (Fig. 3.4). The optimum concentration was 0.2  $\text{ul ml}^{-1}$ , which was biologically equivalent to 1.95  $\mu\text{M}$  of BA. Growth retardation associated with CSE was not found even when CSE concentration was 0.15  $\text{ul ml}^{-1}$  which was 5 times higher than the optimum concentration.

It can be seen in Fig. 3.1, Fig. 3.3, and Fig. 3.4 that cotyledon growth had a different response to the FSE treatment than to either BA or CSE treatment. The growth of FSE-treated cotyledons was inhibited when the FSE concentration was higher than 10  $\text{ul ml}^{-1}$ , while growth in the BA and CSE treated cotyledons was retarded with higher concentrations but still higher than the growth in the control. This implies that FSE may contain some growth inhibitor(s) which retard cell expansion when accumulated to a certain level. Since ABA has been identified in seaweed and SE (Kingman and Moore, 1982; Crouch, 1990), it is possible that ABA may be responsible for the inhibition observed in these experiments. Although the CSE

is a SE, the different extract procedure used in its preparation probably reduced or eliminated the growth inhibitor in CSE.

Both PPC and TRF inhibited cotyledon expansion as concentrations increase (Fig. 3.5 and Fig. 3.6).

The data from this study confirmed that FSE and CSE had growth promoting activity in the cotyledon bioassay for cytokinin. The optimum concentration of FSE and CSE from this bioassay could serve as a basis for determining levels of FSE and CSE application. Furthermore, the results showed that PPC and TRF do not have cytokinin activity in this bioassay. The PPC- and TRF-induced changes in morphology and physiology, such as delayed senescence, darker green color, and increased root growth (Goatley and Schmidt, 1990), may not be directly related to cytokinin activity.

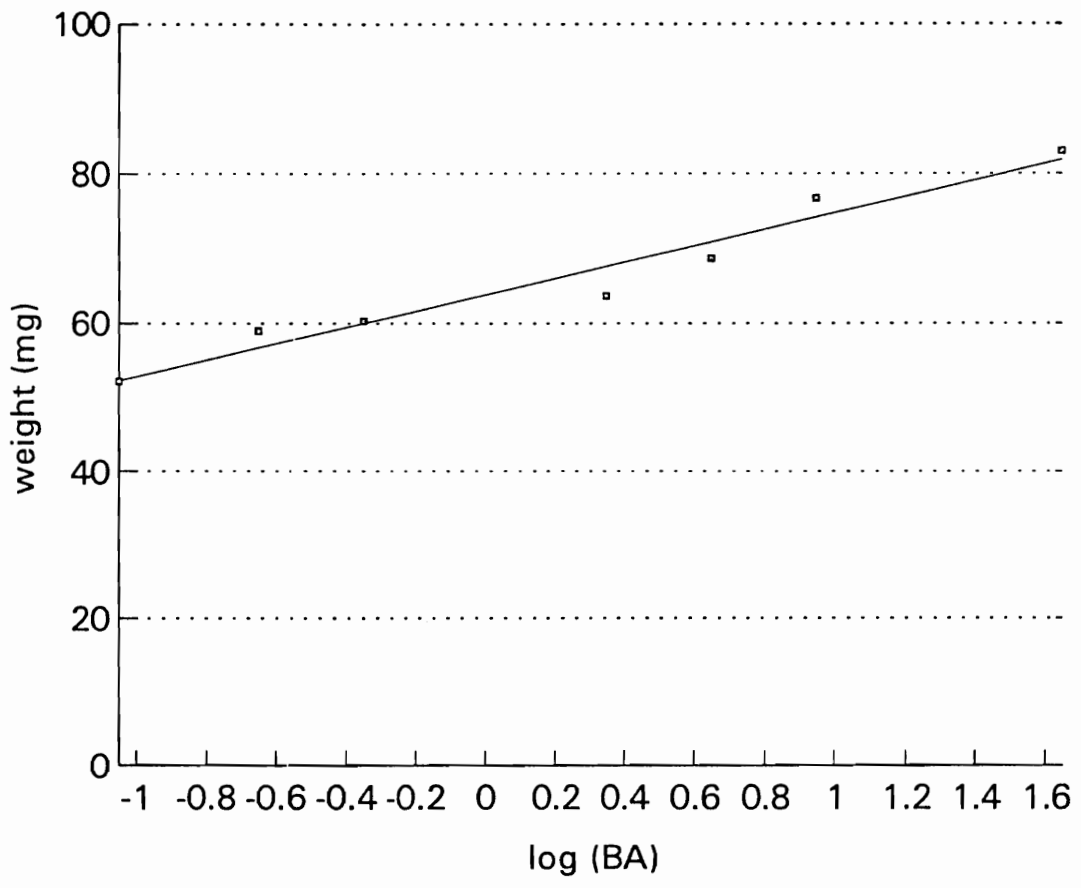
#### **Endogenous Cytokinin in the PGRs-Treated Ryegrass:**

Using the first group of incubation solution, which was extracted from 3 g of PGR-treated ryegrass, a significantly higher cytokinin bioactivity was found only in the incubation solution extracted from FSE-treated ryegrass (Table 3.1). This suggests that FSE treated ryegrass has a higher level of endogenous cytokinin than the control (without any PGR treatment). Endogenous cytokinin activity in CSE, PPC, and TRF treated ryegrass was not significantly different from that in the control (without PGR treatment). However, using the second group of incubation solution, which was diluted 2 times, the higher cytokinin bioactivity was exhibited in the solution extracted from FSE and CSE-treated ryegrass, but not exhibited in the solution extracted from

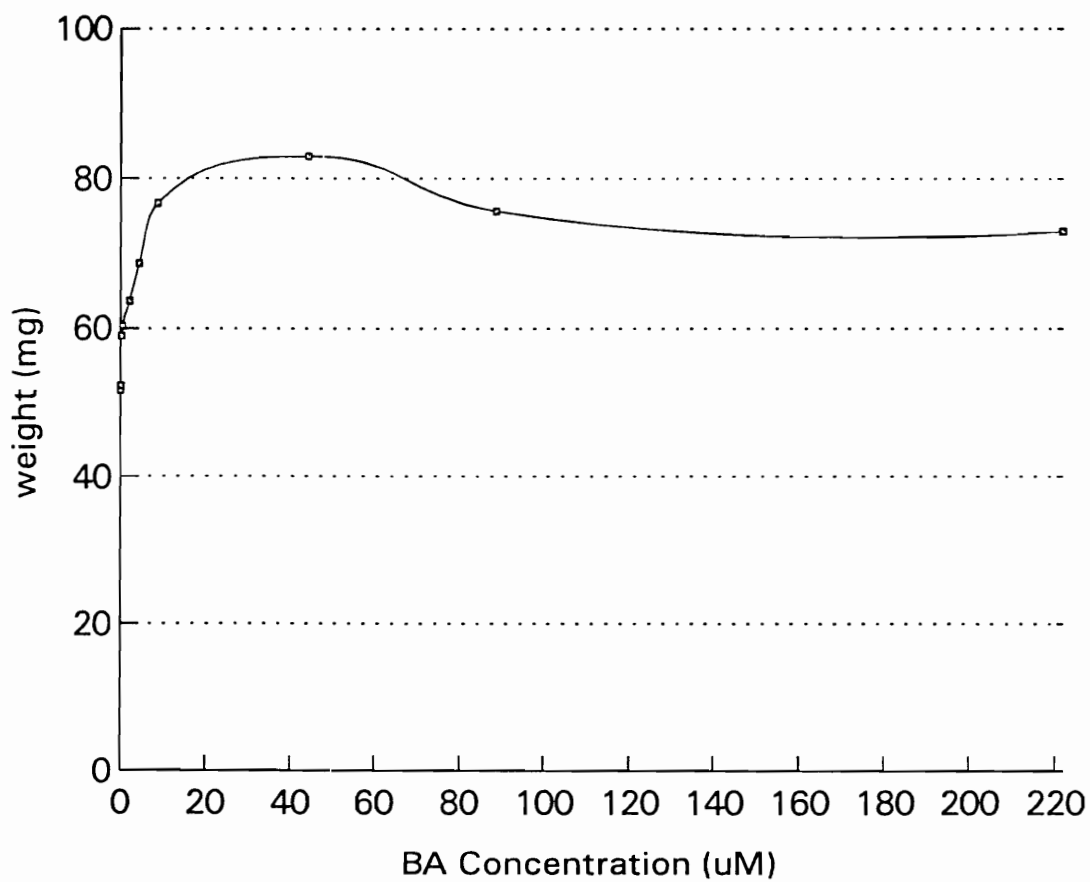
PPC and TRF-treated ryegrass.

This research demonstrated that FSE and CSE may stimulate endogenous cytokinin in ryegrass. However, the high cytokinin bioactivity stimulated by CSE showed only when the extract solution was diluted. This is consistent with the response of cotyledons to exogenous CSE application. Perhaps the CSE either stimulates this substance or increases plant sensitivity to this substance which had its highest cytokinin bioactivity only at a low concentration.

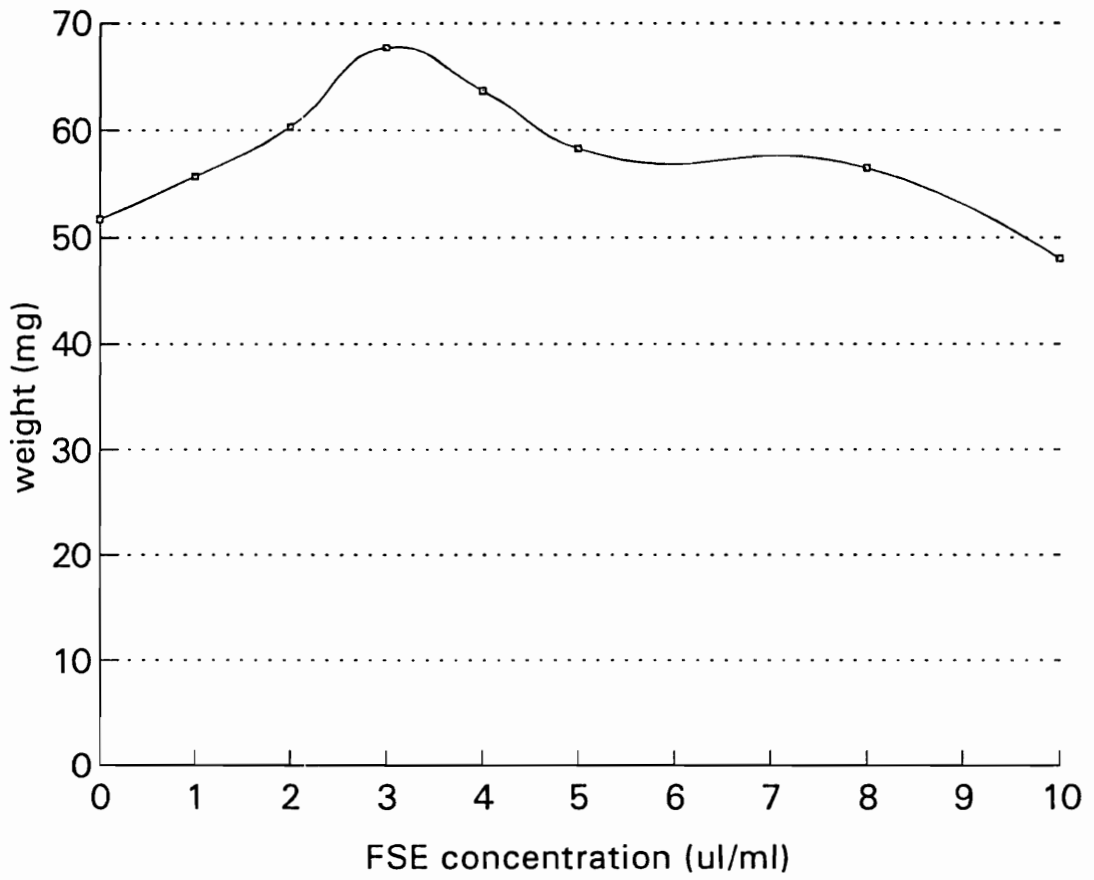
Propiconazole and TRF did not contain cytokinin or cytokinin-like substance. The application of PPC and TRF did not significantly affect endogenous cytokinin activity in ryegrass as determined by the radish cotyledon bioassay.



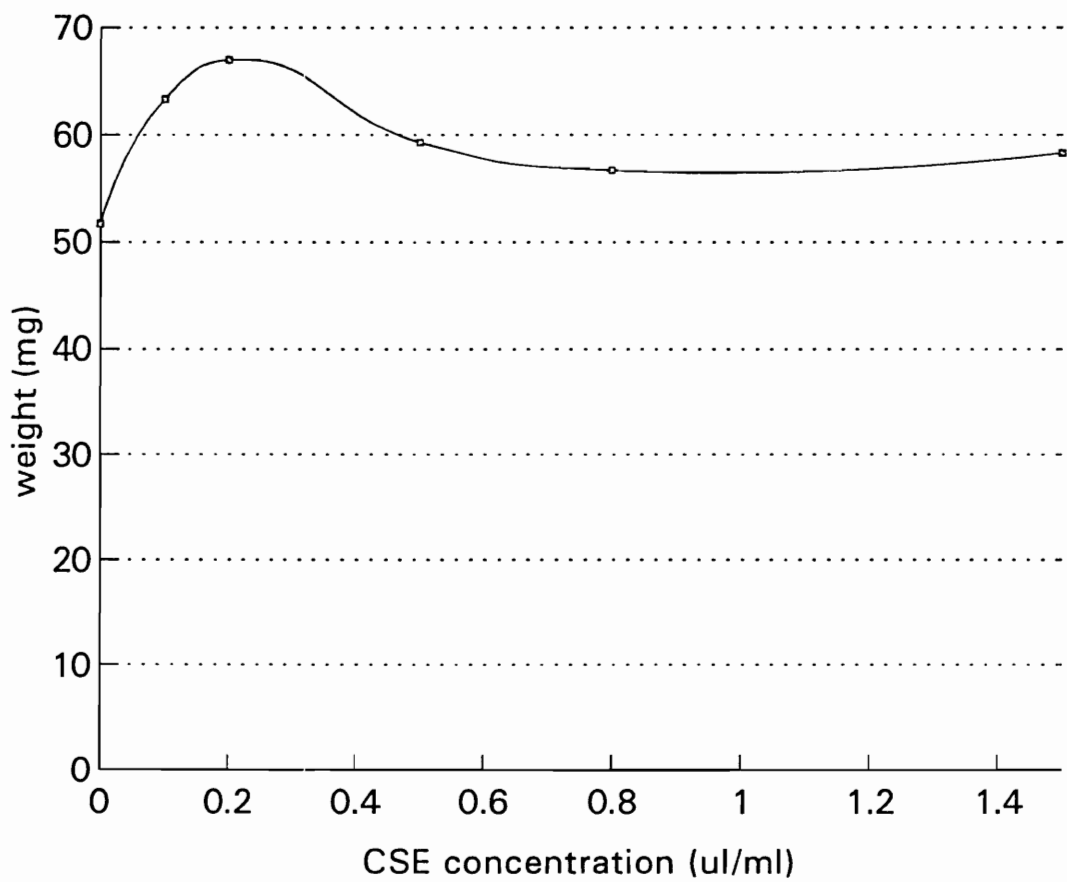
**Figure 3.1. Standard curve showing the linear relationship between logarithmic value of 6-Benzylaminopurine (BA) concentration (uM) and fresh weight of radish cotyledons (mg).**



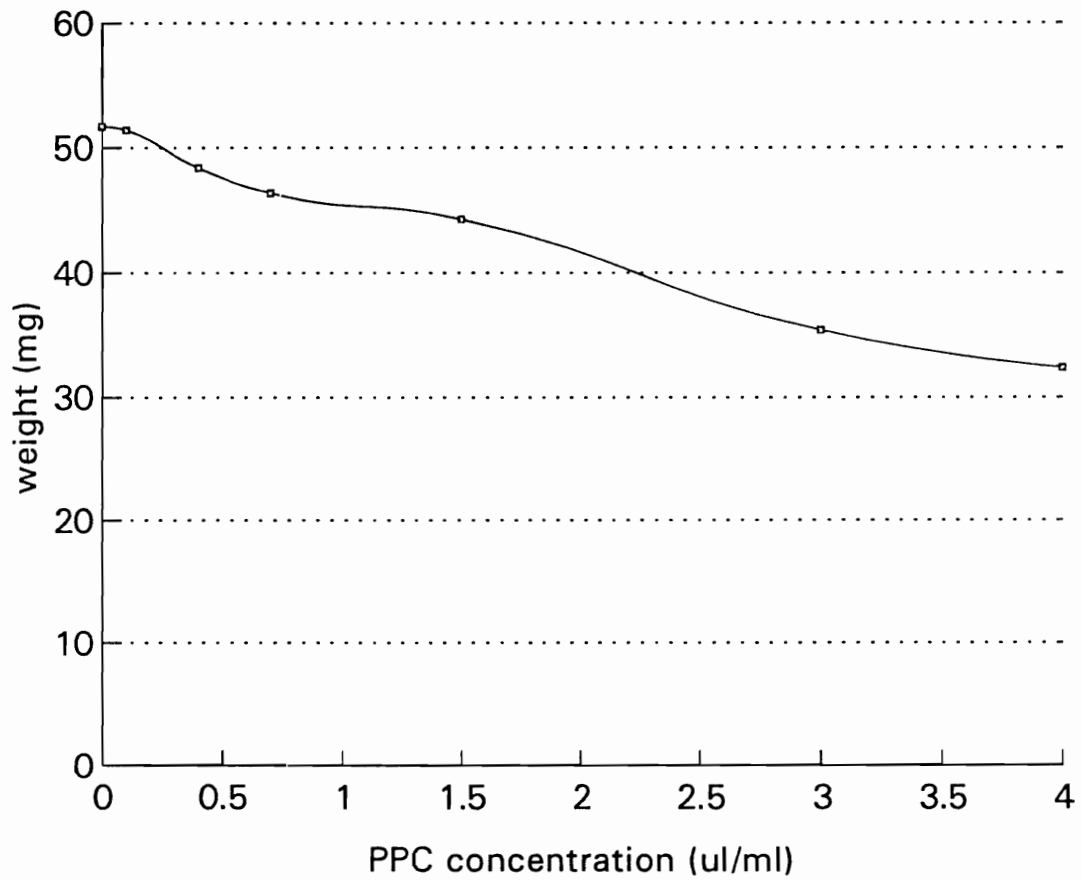
**Figure 3.2. Standard curve showing relationship between 6-Benzylaminopurine (BA) concentration (uM) and fresh weight of radish cotyledons (mg).**



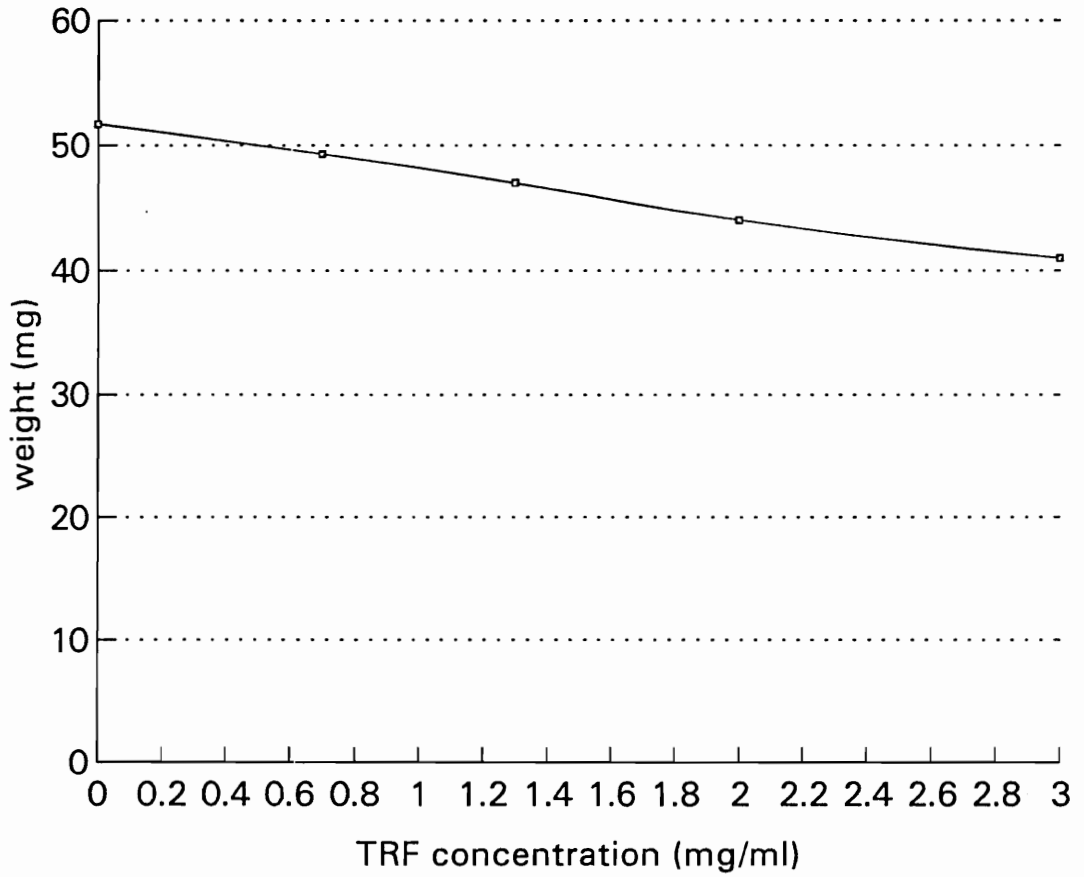
**Figure 3.3. Relationship between concentration of fortified seaweed extract (FSE) ( $\text{ul ml}^{-1}$ ) and fresh weight of radish cotyledons (mg).**



**Figure 3.4. Relationship between concentration of concentrated seaweed extract (CSE) ( $\text{ul ml}^{-1}$ ) and fresh weight of radish cotyledons (mg).**



**Figure 3.5. Relationship between concentration of propiconazole (PPC) ( $\text{ul ml}^{-1}$ ) and fresh weight of radish cotyledons (mg).**



**Figure 3.6. Relationship between concentration of triadimefon (TRF) ( $\text{mg ml}^{-1}$ ) and fresh weight of radish cotyledons (mg).**

**Table 3.1. The fresh weight (mg) of radish cotyledons grown in the endogenous cytokinin solution extracted from perennial ryegrass treated with plant growth regulators (PGRs).**

PGRs	Radish Cotyledon Weight (mg)	
	4 ml endogenous cytokinin extract	2 ml extract + 2 ml buffer
FSE	87.7 a	90.0 a
CSE	62.0 b	67.0 b
PPC	58.0 b	50.5 c
TRF	56.0 b	52.0 c
Control	63.6 b	54.5 c

Fresh weight of radish cotyledons was 51.7 mg in the 4 ml of potassium phosphate buffer solution.

Within columns, means followed by a different letters are significantly different from each other based on LSD at  $\alpha = 0.10$ .

FSE: fortified seaweed extract

CSE: concentrated seaweed extract

PPC: propiconazole

TRF: triadimefon

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## **Chapter 4**

# **Alteration of Ryegrass Polar Lipid Composition Related to Drought Stress and Propiconazol**

### **Introduction**

Propiconazole (PPC) is a synthetic triazole fungicide. Generally, the triazole fungicides disrupt fungal growth and reproduction by inhibiting demethylation of 24-methylenedihydrolansterol at the C-14 position with subsequent blockage of ergosterol synthesis. Ergosterol is a principal sterol in most fungi and an indispensable component of fungal membranes (Koller, 1987; Weete, 1987).

Numerous side effects of triazole fungicides on plant growth and development have been observed, including reduction of shoot and leaf growth; decrease in transpiration and increase in yield under water stress; protection from chilling, heat or ozone injuries; enhancement of cytokinin-like activity with anti-senescence properties; increase in chlorophyll; and decrease in gibberellin content (Fletcher and Hofstra, 1985; Fletcher et al., 1986).

A proposed site of action of triazole chemicals in plants is the oxidation of *ent-*

kaurene to *ent*-kaurenoic acid which is a precursor of gibberellic acid (Koller, 1987). This oxidation reaction is, like sterol demethylation, catalyzed by a specific cytochrome p-450 mixed-function oxygenase (Amrhein, 1983; Rademacher et al., 1984). All triazole chemicals have sp<sup>2</sup>-nitrogen atom with a free electron pair which could bind fungal cytochrome p-450 to inhibit sterol demethylation and bind plant cytochrome p-450 to inhibit the biosynthesis of gibberellin (Marchington, 1984).

It is well known that proper functioning of cell membranes depends upon optimum membrane fluidity, since membrane-associated functions, including enzymes, receptors, and ion channels are modulated by changes in membrane fluidity (Sandermann, 1978; Quinn, 1981). Almost all polar lipids locate in cell membrane. The polar lipid composition and free sterol content are major factors determining membrane fluidity (Shinitzky, 1984; Bishop et al., 1979). In general, the more unsaturated fatty acids of polar lipids are, the more fluid the membrane becomes (Quinn, 1981; Subbs, 1983; Subbs and Smith, 1984). The increase of free sterol content in a membrane could decrease fatty acyl chain mobility and reduce an intermediate fluid state (Stockton and Smith, 1976; Van Dijck et al., 1976). Therefore, a high free sterol content could result in membrane becoming more stable and rigid (Shinitzky, 1984). When plants are exposed to an unfavorable environmental state, membrane fluidity appears to be adjusted through an alteration of lipid composition and sterol level to an optimal state for the changed environment (Cornan and Gelmann, 1975; Hazel and Prosser, 1974). Possibly, it is the capability of plants to regulate their membrane fluidity under stress that determines their adaptation and tolerance to environmental stress.

A number of papers describe the correlation between water stress and changes in membrane lipid composition (Liljenberg et al., 1985; Navari-Izzo et al., 1989; Svenningsson and Liljenberg, 1986). Differences of membrane lipid composition between a drought-resistant variety and drought-sensitive variety have been reported for *Vigna unguiculata* L. (Pham Thi et al., 1989) and for Durum wheat (*Triticum durum*) (Kamell and Losel, 1989).

Triazole fungicides have been reported to have a significant impact on membrane lipid composition and cell membrane function in fungi, including an increase of membrane lipid fatty acid content and a shift of fatty acid to higher degree of unsaturation (Sisler and Ragsdale, 1984). However, little has been reported concerning the influence of the triazole fungicides on higher plant cell membrane composition. It will be valuable to ascertain whether PPC increases drought tolerance in plants through its influence on membrane lipid composition.

The objectives of this study were to determine the effect of PPC and drought stress on cell membrane lipid composition in ryegrass and to ascertain the relationship between drought tolerance and the regulatory function of PPC.

## **Materials and Methods**

**Plant culture and treatment:** One hundred sixty grams of Perennial ryegrass (*Lolium perenne* L.) seeds were soaked in 100 ml of 0.03% PPC solution for 10 minutes, and then were dried overnight. Three grams of the treated seeds were sown

into 1 liter plastic pots with holes in the bottom. Each pot had the same amount of sandy soil 1,000 g. When the seedlings were two weeks old, 10 ml of PPC solution (0.03%) were sprayed by syringe on the foliage of each pot.

Drought stress treatment begun on 3-week-old seedling. One hundred ml of water was applied twice a week for drought-treated ryegrass pots, whereas 200 ml of water was applied twice a week for well-watered ryegrass pots, until the grass was eight weeks old when the drought-treated control ryegrass (without PPC treatment) was very wilting.

**Leaf water potential measurement:** A series of ryegrass leaves with different drought statue were used. Leaf water potential (LWP) and water stress level (WSL) in the ryegrass leaves were determined by pressure bomb and hydraulic press (Decagon Devices Inc, Pullman, WA.) (Cox and Hugher, 1982) respectively at the same time. The relationship between LWP and WSL can be expressed in the regression formula:  $LWP = 3.24 e^{-0.0145 X}$  ( $r^2 = 0.9877$ ), where X is WSL measured by hydraulic press, and LWP data was measured by pressure bomb. The average of WSL in 5 piece of leaves treated with or without PPC and drought stress was measured by hydraulic press right before the harvesting, and then was converted to LWP value by the formula above.

Each treatment was replicated three times and randomly placed in a greenhouse. Final results were analyzed by ANOVA and means were separated with LSD tests.

**Total Lipid Extraction:** Five hundred mg of fresh leaf tissues were frozen and crushed in liquid nitrogen, extracted with 5 ml  $CHCl_3 : MeOH : H_2O$  (1:2:0.8, v/v/v)

one time and with  $\text{CHCl}_3$  three times. Between each extraction, the residue was pelleted by centrifugation. One ml of 1% NaCl solution was added to the extract to make phase separation, and then centrifuged for 10 minutes at 3000 rpm. Total lipids in the bottom layer ( $\text{CHCl}_3$ ) were transferred with pipet into a pre-weighed test tube, were dried under the  $\text{N}_2$  at 40 °C, and weighed.

**Polar Lipid Extraction and Fatty Acid Analysis:** The total lipids were dissolved in 500 ul of  $\text{CHCl}_3$ , and 200 ul of them were spotted and separated on silica gel (Kiesel gel, 60 g, EM Science, Elmsor NY) thin layer chromatograph (TLC) plates (0.5 mm thickness, 15 X 15  $\text{cm}^2$ ) using a solvent system of hexane:diethyl ether:acetic acid (80:20:1, v/v/v). Polar lipids were remained at origin of the TLC plate, were visualized with 0.2% 2,7-dichlorofluorescein in 95% ethanol under long wave ultraviolet light, and then removed from the TLC plates. The polar lipids were eluted from the silica gel with  $\text{CHCl}_3$ :MeOH (2:1, v/v) and diethyl ether, and dried under  $\text{N}_2$ . Heptadecanoic acid ( $\text{C}_{17}$ ) was added as an internal standard into the polar lipid sample just before the elution from the silica gel. Lipids were hydrolyzed with 5% KOH in 80% MeOH at 55 °C for 45 min and methylated with  $\text{BCl}_3$ :MeOH at 55 °C for 5 min. The methylated fatty acids were rechromatographed with TLC described previously. The standard methylated  $\text{C}_{17}$  was spotted at the TLC to identify the methylated fatty acids. The methylated fatty acids were eluted from silica gel with hexane. Gas chromatography (GC) analysis was performed using a Bendix 2500 GC (Bendix Process Instrument Division, Ronceverte, WV) as described as Goedhart (1989). A 2m x 4mm i.d. glass column packed with 10% DEGS on 80/100

chromasorb WAW was used for separating fatty acids at a column temperature of 195 °C. The fatty acids were identified by comparison of GC retention times with known standards, and were quantified by comparison with known amounts of internal standards.

**Sterol Identification and Quantification:** Rest of 300 ul total lipids was dried and dissolved in 400 ul of CHCl<sub>3</sub>. One ul of them was spotted on a high performance thin layer chromatograph (HPTLC) plate (10 cm x 10 cm) which was prewashed twice in CHCl<sub>3</sub>:MeOH:H<sub>2</sub>O (65:25:0.4, v/v/v) (Orcutt and Hopkins, 1990). Two solvent systems were used for sterols separation. First solvent was ether:hexane:acetic acid (35:65:2, v/v/v) which was allowed to migrate to 7.5 cm above the origin line. The HPTLC plate was developed twice in the first solvent. The second solvent was CHCl<sub>3</sub>:MeOH:H<sub>2</sub>O (85:15:0.5, v/v/v) which was allowed to migrate to 2.5 cm above the origin. The plates were dried and then dipped in a solution of 3% copper acetate in 8% phosphoric acid for 8 seconds. Then the plates were dried and charred at 150 °C for 5 min to visualize the sterols, then scanned with Camag ScannerII densitometer (Camag Scientific Inc, Wrightsvill Beach NC) at 440 nm. The standards cholesterol, lanosterol, and cholesterol oleate were used to identify and quantify the demethyl sterol (DeMS), dimethyl sterol (DiMS), and steryl ester (STE) respectively. The concentration range of these sterols was from 0.25 mg ml<sup>-1</sup> to 1.5 mg ml<sup>-1</sup>. The concentration ranges of standards steryl glycosides (SG) and esterified steryl glycosides (ESG) were from 0.05 mg ml<sup>-1</sup> to 0.25 mg ml<sup>-1</sup> respectively. Standard curves were generated for each HPTLC plate.

## Results

Total lipid concentration in control ryegrass (without PPC treatment) was 118 mg g<sup>-1</sup> dw under well-watered conditions, and dropped to 87.65 mg g<sup>-1</sup> dw when subjected to drought stress. Total lipid concentration under drought stress did not significantly decrease in PPC-treated ryegrass (Table 4.1). Ryegrass treated with PPC had a higher concentration of total lipids than the control ryegrass under both well-watered (142.73 mg g<sup>-1</sup> dw) and drought stressed conditions (122.87 mg g<sup>-1</sup> dw) (Table 4.1).

The ryegrass treated with PPC had lower concentration of total polar lipid fatty acids (TPLFA) than the control under well-water conditions. Drought stress treatment resulted in a decrease in TPLFA in control ryegrass from 33.8 mg g<sup>-1</sup> dw to 15.88 mg g<sup>-1</sup> dw, but resulted in an increase in PPC-treated ryegrass from 17.6 mg g<sup>-1</sup> to 22.56 mg g<sup>-1</sup> dw (Table 4.1).

Linolenic acid (C<sub>18:3</sub>) was a major component in the cell membrane lipids of ryegrass and accounted for about 80% of total PL fatty acids. Linoleic acid (C<sub>18:2</sub>) and palmitic acid (C<sub>16:0</sub>) were the other two most abundant important fatty acids in the ryegrass. Drought stress did not significantly change the proportion of each PL fatty acid in the control ryegrass (Table 4.2). However, PPC-treated ryegrass had less unsaturated fatty acid (C<sub>18:3</sub>) and more saturated fatty acid (C<sub>16:0</sub>, C<sub>14:0</sub>) than the control under well-watered conditions; and by contrast, had more unsaturated fatty acid (C<sub>18:3</sub>) and less saturated fatty acid (C<sub>16:0</sub>, C<sub>14:0</sub>) than the control under drought stress conditions (Table 4.2). The change in unsaturation of the PL fatty acids could

be expressed as the double bond index (DBI). DBI represents the degree of unsaturation of polar lipids, which is equal to the product of the percentage of each PL fatty acid and the number of double bonds in the PL fatty acid (Bishop, 1979). The DBI was lower in PPC treated grass under well-watered conditions, and then drastically increased through drought stress treatment (Table 4.3). There was little change in DBI in control ryegrass after drought stress (Table 4.3).

Drought stress caused a decrease in DeMS concentration, and thus a decrease in total free sterols (TFS) in control ryegrass, but had little effect on TFS in PPC-treated ryegrass (Table 4.4). The PPC-treated ryegrass had more DiMS and less DeMS than the control ryegrass under well-watered conditions. Steryl ester was a predominant conjugated sterol in ryegrass and accounts for about 50% of the total conjugated sterols (TCS). The STE concentration increased by PPC treatment, but was affected little by drought stress either in control or in PPC-treated ryegrass.

The ratio of free sterols to polar lipids (FS/PL) was influenced by both PPC treatment and drought stress (Table 4.3). The PPC-treated ryegrass exhibited higher FS/PL than control ryegrass; however, drought stress caused FS/PL to increase in control but decrease in PPC-treated ryegrass (Table 4.3).

The ryegrass treated with PPC had higher leaf water potential than control ryegrass under both well-watered and drought stress conditions. The difference in leaf water potential between PPC treated and control ryegrass was more distinct under drought stress (Table 4.3).

**Table 4.1. Total lipids and total polar lipid fatty acids in leaves of perennial ryegrass treated with or without propiconazole and drought stress.**

Treatment	Total Lipids	TPLFA
----- mg g <sup>-1</sup> dry wt -----		
well-watered		
Control	118.00 b	33.80 a
PPC	142.73 a	17.60 c
drought stress		
Control	87.65 c	15.88 c
PPC	122.87 a	22.56 b

Within columns, means followed by different letters are significantly different based on LSD at  $\alpha = 0.1$ .

TPLFA = total polar lipid fatty acids.

PPC = propiconazole.

**Table 4.2. Polar lipid fatty acid concentration in leaves of perennial ryegrass treated with or without propiconazole and drought stress.**

Treatment	C <sub>14:0</sub>	C <sub>14:1</sub>	C <sub>16:0</sub>	C <sub>18:0</sub>	C <sub>18:2</sub>	C <sub>18:3</sub>
----- % of total -----						
well-watered						
Control	0.53 b	0.50 b	13.55 b	1.05 b	4.49 a	79.21 b
PPC	2.14 a	1.99 a	16.86 a	3.14 a	4.51 a	71.37 c
drought stress						
Control	1.05 a	0.30 b	13.48 b	1.40 b	4.71 a	78.58 b
PPC	0.43 b	0.33 b	11.94 b	1.34 b	4.01 a	81.95 a

Within columns, means followed by different letters are significantly different based on LSD at  $\alpha = 0.1$ .

PPC = propiconazole.

**Table 4.3. Double bond index, leaf water potential, and ratio of free sterols to polar lipids in leaves of perennial ryegrass treated with or without propiconazole and drought stress.**

Treatment	DBI	FS/PL	LWP(MPa)
		well-watered	
Control	2.48 b	0.11 c	-0.47 b
PPC	2.23 c	0.23 a	-0.36 a
		drought stress	
Control	2.45 b	0.17 b	-1.52 d
PPC	2.54 a	0.17 b	-0.55 c

Within columns, means followed by a different letters are significantly different based on LSD at  $\alpha = 0.1$ .

DBI = double bond index =  $\Sigma$  ( % of each fatty acid X double bond numbers in the fatty acid).

FS/PL = ratio of free sterols to polar lipid fatty acids.

LWP = leaf water potential

PPC = propiconazole.

**Table 4.4. Free sterols and conjugated sterols in leaves of perennial ryegrass treated with or without propiconazole and drought stress.**

Treatment	Free Sterols			Conjugated Sterols			
	DiMS	DeMS	Total	SG	ESG	STE	Total
----- mg g <sup>-1</sup> dry wt -----							
well-watered							
Control	1.78 a	2.06 a	3.84 a	1.61 a	0.45 a	5.51 b	7.57 a
PPC	2.4 a	1.66 a	4.06 a	0.67 b	0.53 a	7.59 a	8.79 a
drought stress							
Control	1.46 a	1.21 b	2.67 b	1.47 a	0.30 a	4.37 b	6.14 a
PPC	2.26 a	1.65 a	3.91 a	1.40 a	0.69 a	6.55 a	8.64 a

Within columns, means followed by a different letters are significantly different based on LSD at  $\alpha = 0.1$ .

DiMS = dimethyl sterols.

DeMS = demethyl sterols.

SG = steryl glycosides.

ESG = esterified steryl glycosides.

STE = steryl ester.

PPC = propiconazole.

## Discussion

Total lipids extracted with  $\text{CHCl}_3$  : MeOH :  $\text{H}_2\text{O}$  could have a complex composition, including wax, vitamins, growth substances, terpenes, etc. (Lehninger, 1975). These components may play important roles in drought tolerance. For example, wax on leaves could resist evapotranspiration and reduce water loss. In this study, high water potential (Table 4.3) and high drought tolerance were consistent with the high content of total lipids (Table 4.1) in the treated ryegrass. However the effects of PPC on wax and other substances cited above were not the focus of this study.

Proper membrane fluidity is necessary for proper physiological and biochemical functions of plant cells. Membrane fluidity can be adjusted to an optimal state via alteration of unsaturation, length, and composition of fatty acids groups of polar lipids. Changes in free and conjugated sterol ratios may change the availability of free sterols for incorporation into membranes, thus affecting fluidity (Shinitzky, 1984).

Norberg and Liljenberg (1991) found that oat roots showed drought tolerance along with changing of phospholipid concentration and free sterol levels after repeated periods of drought stress. The phospholipid concentration was lower in a drought-resistant variety than in a drought-sensitive variety of Durum wheat. However, after drought stress treatment, the phospholipid content was increased in the drought-resistant variety and decreased in the drought-sensitive variety (Kamell and Losel, 1989). Similar results were found in cotton leaves between drought-resistant and drought-sensitive varieties (Pham Thi, 1989).

In this study, changes in membrane lipid composition in PPC treated ryegrass are consistent with the changes observed in phospholipids of drought-tolerant varieties under drought stress. Under the well-watered conditions, TPLFA concentration and DBI in PPC-treated ryegrass was lower (Table 4.1). The lower TPLFA concentration was accompanied by lower unsaturation of polar lipids and lower membrane fluidity.

When water supply is sufficient and cell is turgid, the less fluid membrane, or less elastic membrane, could accompany a higher turgor pressure, therefore increase leaf water potential.

Under drought stress, PPC-treated ryegrass exhibited an increase in unsaturation of membrane lipids via the increase of unsaturated fatty acid concentration and proportion (Table 4.1, 4.3). This change, like the change in membrane lipid composition in drought-resistant wheat and cotton mentioned above, could be an adaption response to the drought stress environment.

Earlier responses of plants to drought stress are stomatal closure and inhibition of growth. These responses are all directly caused by a decrease in cell turgor (Levitt, 1980). With a decrease of cell turgor, growth (cell enlargement) is inhibited; stomata are closed; CO<sub>2</sub> and O<sub>2</sub> flow are retarded; photosynthesis and respiration are inhibited; proteins are broken down; toxic products are accumulated; enzymes are inactivated; and finally, plant senescens and dies. However changes in cell turgor may depend upon plasma membrane fluidity and permeability to water and solutes (Levitt, 1980).

When plant is in the beginning of water-deficit conditions, perhaps its rigid membrane might prevent loss of solutes and water from the cells. However, with the

more serious water-deficit, the rigid membrane may become disadvantage. On the contrary, increase of membrane fluidity, which increases membrane permeability to water and solutes, could enhance the absorption and translocation of osmotic substances, and improve osmotic adjustment to maintain high turgor. Furthermore, the increase of membrane fluidity makes membranes more elastic and flexible (Norberg and Liljenberg, 1991), these properties would prevent cell turgor from dropping down rapidly when cells lose water under drought stress. This may explain why a higher LWP in PPC-treated ryegrass was coincident with higher unsaturation of membrane lipids (DBI) and decreased FS/PL ratio under drought stress treatment. An increase in membrane fluidity, as reflected by an increase in the double bond index of polar lipids in a plant under drought stress, could be related to increased drought tolerance. In the current study, the control ryegrass (without PPC treatment) showed little change in DBI, which may indicate little changes in membrane fluidity and related less tolerance to drought stress. However, the total free sterols (TFS) was accumulated more in PPC-treated ryegrass than that in control under drought stress, which might modify the change of membrane fluidity resulting from fatty acid changes. However, the mechanism by which PPC affects membrane lipid composition is as yet not known precisely, and further research is needed.

Higher ratios of DiMS to DeMS in PPC treated ryegrass (Table 4.4) could result from the sterol demethylation inhibition of PPC. This inhibition is lethal for fungi but not for higher plants, because fungi and plants utilize sterols differently. The fungal ergosterol may not be indispensable in plant.

Steryl ester (STE) is the most efficient storage sterol for interconversion with free

sterols (Mudd, 1980). The PPC-treated ryegrass had a higher level of STE (Table 4.4), which indicates that PPC-treated ryegrass had an effective way to adjust membrane sterol levels and to balance membrane fluidity.

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## **Chapter 5**

# **Effects of Fortified Seaweed Extract on Polar lipid Composition and Drought Tolerance in Ryegrass**

### **Introduction**

Seaweed extracts (SE) have been used for many centuries, since they are beneficial to plant growth and development. They are known to increase plant vigor and yield (Crouch, 1990), stimulate root growth, delay senescence, maintain green color, and improve resistance to environmental stress (drought, salt, temperature, etc.) (Schmidt, 1990; Goatley and Schmidt, 1990; Crouch, 1990). However the physiological mechanism and roles of SE in plants is still under investigation.

Initially, SE was used as a soil conditioner to improve soil structure for root growth (Milton, 1964). However, when SE was used in small amounts and applied to foliage, the amount of SE in the soil can not be considered significantly enough to have effects on soil structure (Crouch, 1990). It was also thought that trace elements in seaweed produced these beneficial effects on the SE-treated plants (Francki, 1960). But later, the concentration of the trace elements in SE was determined and considered too low to have any significant effects on plant growth (Abetz, 1980;

Blunden, 1977). Recently, more and more research has demonstrated that most of the benefits associated with SE applications can be attributed to plant growth substances in SE. Cytokinin or cytokinin-like substances have been identified in all commercial SE (Bently-Mowat and Reid, 1968; Hussian and Boney, 1969; Mooney and Van Staden, 1986; Kentzer et. al., 1980; Brain et al., 1973). The other growth substances, such as auxin-like substances (Williams et. al., 1976; Sanderson and Jameson, 1986; Kingman and Moore, 1982; Sumera and Cajipe, 1981), gibberellin-like substances (Mowat, 1963, 1965; Jennings, 1968; Taylor and Wilkinson, 1977; Wildgoose et al., 1978), and abscisic acid-like substances (Hussian and Boney, 1973; Kingman and Moore, 1982) were detected in seaweed and in SE. Nevertheless, the cytokinin-like substance was determined to be the most active component in SE (Hester, 1984). Most plants responded similarly when treated with SE and cytokinins (Schmidt, 1990; Crouch, 1990).

As early as 1944, Veldstra suggested that changes in membrane permeability to water and solutes could be one of the principal effects of growth substances. Since then there have been many studies demonstrating the effects of growth substances (cytokinin, GA, auxin, ABA, and ethylene) on membrane permeability (Feng, 1973; Humphreys and Dugger, 1957; Ilan, et al. 1971; Tomizawa, 1956; Glinka, 1971). Basically, all these growth substances can affect membrane permeability. However, there was wide variation in these investigations, since the research both on cell membranes and on growth substances are complicated and limited.

The cell membrane is most sensitive to environmental stresses. Specifically, polar lipids (PL), the major component of membranes, can be altered in their composition

when plants are subjected to a changing environment (Shinitzky, 1984). The alteration of membrane lipids directly influences membrane fluidity and permeability (Quinn, 1981; Subbs, 1983; Bishop et al., 1979). Different alterations of membrane lipids were found in drought-resistant and -susceptible cultivars under water deficit conditions (Pham Thi et al., 1989; Kamell and Losel, 1989; Pillay and Beyl, 1990). Alteration of phospholipids and sterols has been observed in oat root (Norberg and Liljenberg, 1991; Liljenberg et. al., 1985), maize seedling (Navari-Izzo et. al., 1989), and rape root (Svenningsson and Liljenberg, 1986) when these plants were subjected to water deficits.

Enhancement of drought tolerance has been observed in SE treated turfgrass (Nabati, 1991). However, it is not clear whether this enhancement is the effect of SE on membrane lipids or which component is most responsible for the enhancement. Answering these questions would help us to understand the regulation mechanism of SE on drought tolerance and to find the function of growth substances in stress physiology.

The objectives of this study were to determine effects of the fortified seaweed extract (FSE) on ryegrass cell membrane lipids and to ascertain the relationship between drought tolerance and the effect of FSE on cell membrane lipids.

## **Materials and Methods**

**Fortified seaweed extract application and plant culture:** Fortified seaweed extract

(FSE) supplied by ROOTS Inc (New Haven, CT) is composed of the extracts of eight seaweeds and is fortified with humic acids and vitamins B and C (see chapter 3, materials and methods section for detail).

One hundred sixty grams of perennial ryegrass (*Lolium perenne* L.) seeds were soaked in 100 ml of 0.3% FSE solution for 10 minutes. Then the treated seeds were dried over night, and 3 g were sown in 1 L plastic pots filled with 1,000 g of sandy soil. When the seedlings were 2-week old, 10 ml of 0.3% FSE were sprayed on the foliage with a plastic syringe.

Drought stress treatment and leaf water potential (LWP) measurement were processed as same as described in Chapter 4 (materials and methods section).

After harvesting, the total lipids and polar lipids extraction, polar lipid fatty acid analysis, and sterols analysis were processed as described in Chapter 4.

## Results

The total lipid (TL) concentration in FSE-treated and control ryegrass leaves were not significant different either in the well-watered or drought stressed conditions (Table 5.1). However, drought stress resulted in a decrease of TL about 25% in both FSE-treated and control ryegrass (Table 5.1).

The concentration of total polar lipid fatty acids (TPLFA) was significantly lower in FSE-treated ryegrass than that in the control under well-watered conditions. Drought stress caused a decrease of TPLFA 53% in control ryegrass and 48% in

FSE-treated ryegrass (Table 5.1). However, there was no significant difference of TPLFA between FSE-treated and control ryegrass under drought stress.

Linolenic acid ( $C_{18:3}$ ) was a major fatty acid and made up 80% of TPLFA. Linoleic acid ( $C_{18:2}$ ) and palmitic acid ( $C_{16:0}$ ) were two abundant fatty acids influencing unsaturation of membrane lipids. Under well-watered conditions, FSE-treated ryegrass tended to have a lower percentage of unsaturated fatty acids ( $C_{18:3}$  and  $C_{18:2}$ ), and a higher percentage of saturated fatty acids ( $C_{18:0}$  and  $C_{14:0}$ ) than control ryegrass (Table 5.2). Under drought stress, the percentage of the unsaturated fatty acid ( $C_{18:3}$ ) tended to be higher in FSE-treated ryegrass than in the control ryegrass; while the percentage of  $C_{14:0}$  and  $C_{18:0}$  was lower than that in the control ryegrass (Table 5.2). These shifts are expressed as a double bond index (DBI), which represents the degree of unsaturation of polar lipid fatty acids. The DBI was 2.44 and 2.48 in FSE-treated ryegrass and in control respectively under well-watered conditions, and 2.50 and 2.45 in FSE-treated ryegrass and in the control respectively under drought stress (Table 5.3). The significant increase of DBI was only found in the FSE-treated ryegrass when through the drought stress (Table 5.3).

Ratio of free sterols to polar lipids (FS/PL) was increased by FSE treatment. After drought stress treatment, the FS/PL increased in both FSE-treated and control ryegrass, and it was still higher in FSE-treated ryegrass than in control (Table 5.3).

Significantly higher leaf water potential (LWP) was exhibited in the FSE-treated ryegrass than the control ryegrass (without FSE treatment) under both well-watered and drought stress conditions (Table 5.3).

Total free sterols (TFS) (DeMS and DiMS) concentration significantly increased

in the FSE-treated ryegrass compared with the control under both well-watered and drought-stressed conditions (Table 5.4). The drought-induced decrease of TFS was found both in FSE-treated and control ryegrass, which was mainly the result of a decrease in DeMS. The DeMS concentration decreased 48% in FSE-treated ryegrass and 41% in control ryegrass through drought stress. No significant difference in the concentration of total conjugated sterol (TCS) (SG, ESG, and STE) was apparent between FSE-treated and control ryegrass, neither of them in both FSE-treated and control ryegrass was affected by drought stress (Table 5.4).

**Table 5.1. Total lipids and total polar lipid fatty acids in leaves of perennial ryegrass treated with or without fortified seaweed extract and drought stress.**

Treatment	Total Lipids	TPLFA
	----- mg g <sup>-1</sup> dry wt -----	-----
	well-watered	
Control	118.00 a	33.80 a
FSE	132.37 a	30.38 b
	drought stress	
Control	87.65 b	15.88 c
FSE	98.03 b	15.80 c

Within columns, means followed by a different letters are significantly different based on LSD at  $\alpha = 0.1$ .

TPLFA = total polar lipid fatty acid.

FSE = fortified seaweed extract.

**Table 5.2. Polar lipid fatty acid concentration in leaves of perennial ryegrass treated with or without fortified seaweed extract and drought stress.**

Treatment	C <sub>14:0</sub>	C <sub>14:1</sub>	C <sub>16:0</sub>	C <sub>18:0</sub>	C <sub>18:2</sub>	C <sub>18:3</sub>
----- % -----						
well-watered						
Control	0.53 b	0.50 b	13.55 a	1.05 ab	4.49 a	79.21 a
FSE	1.27 a	1.22 a	13.79 a	1.37 a	3.65 a	78.70 a
drought stress						
Control	1.05 a	0.30 b	13.48 a	1.40 a	4.71 a	78.58 a
FSE	0.42 b	0.21 b	13.74 a	0.86 b	4.04 a	80.72 a

Within columns, means followed by a different letters are significantly different based on LSD at  $\alpha = 0.1$ .

FSE = fortified seaweed extract.

**Table 5.3. Double bond index, ratio of free sterols to polar lipids and leaf water potential in leaves of perennial ryegrass treated with or without fortified seaweed extract and drought stress.**

Treatment	DBI	FS/PL	LWP (MPa)
		well-watered	
Control	2.48 ab	----- 0.11 c	-0.47 b
FSE	2.44 b	0.17 b	-0.40 a
		drought stress	
Control	2.45 ab	----- 0.17 b	-1.52 d
FSE	2.50 a	0.21 a	-1.16 c

Within columns, means followed by a different letters are significantly different based on LSD at  $\alpha = 0.1$ .

DBI = double bond index =  $\Sigma$  ( % of each fatty acid X double bond number in the fatty acid).

FS/PL = ratio of free sterols to polar lipid fatty acids.

LWP = leaf water potential.

FSE = fortified seaweed extract.

**Table 5.4. Free sterols and conjugated sterols in leaves of perennial ryegrass treated with or without fortified seaweed extract and drought stress.**

Treatment	Free Sterols			Conjugated Sterols			
	DiMS	DeMS	Total	SG	ESG	STE	Total
----- mg g <sup>-1</sup> dry wt -----							
well-watered							
Control	1.78 b	2.06 a	3.84 b	1.61 a	0.45 a	5.51 a	7.57 a
FSE	2.70 a	2.40 a	5.10 a	1.51 a	0.67 a	5.40 a	7.58 a
drought stress							
Control	1.46 b	1.21 b	2.67 c	1.47 a	0.30 a	4.37 a	6.14 a
FSE	2.18 a	1.24 b	3.42 b	0.75 a	0.29 a	5.31 a	6.35 a

Within columns, means followed by a different letters are significantly different based on LSD at  $\alpha = 0.1$ .

DiMS = dimethyl sterols.

DeMS = demethyl sterols.

SG = steryl glycosides.

ESG = esterified steryl glycosides.

STE steryl ester.

FSE = fortified seaweed extract.

## Discussion

The polar lipids (most of which are phospholipids) are major components of the cell membrane (Lehninger, 1975). Different concentration of polar lipids and double bonds in each polar lipid fatty acid could directly influence membrane fluidity and permeability to water and solutes. Generally, more unsaturated fatty acids of polar lipids are, the more fluid and more permeable the membrane (Shinitzky, 1984). In this study, about 80 - 85% of the TPLFA in the ryegrass leaves were the unsaturated fatty acids  $C_{18:3}$  and  $C_{18:2}$ . So decrease in TPLFA concentration could imply a decrease in membrane lipid unsaturation, and therefore a decrease in membrane fluidity and permeability. Under well-watered conditions, plant cells have enough water and are very turgid. The less fluid and more rigid membrane is less elastic, thus which could cause a higher turgor pressure and higher LWP. The less permeable membrane also benefit plants under mild water deficit. Norberg and Liljenberg (1991) and Liljenberg et al. (1985) found that moderate water-deficiency caused increase of free sterols from 9% to 14% of total sterols and increase of ratio of sterol to phospholipid in oat root cells. But when plant is subjected to a severe water-deficiency conditions, the less permeable and more rigid membrane may become disadvantageous, since it may limit the osmotic adjustment. A more permeable membrane could increase penetration and translocation of water and solutes, accelerate the osmotic adjustment, thereby reduce water loss and enhance water uptake. In addition, the more fluid membrane could be more flexible and

elastic. When water is lost from the cell and the cell shrinks, the flexible membrane could follow this shrinkage by increased curvatures, which is crucial to prevent turgor pressure from further declining and to maintain a relatively higher water potential.

The data from this study supported these hypotheses. The FSE-treated ryegrass had higher water potential both under well-watered and drought stress conditions. The DBI of FSE-treated ryegrass, which represents membrane fluidity, was lower under well-watered conditions and higher under drought stress conditions than the control. The rise of DBI in FSE-treated ryegrass through the drought stress may reflect the adaptation of FSE-treated ryegrass to drought stress.

Moreover, the level of free sterols is another factor determining membrane fluidity. Free sterols are linked between polar lipids. The linkage could decrease the mobility of fatty acyl chains (Cooper, 1978), and decrease membrane fluidity (Stockton and Smith, 1976; Van Dijck et al., 1976). Significantly higher free sterol level in FSE-treated ryegrass under well-watered condition could make the cell membrane less fluid, thus improving cell turgor potential. However, under drought stress, free sterol content in FSE-treated ryegrass significantly decreased, thereby increased the membrane fluidity and permeability. However, FS/PL ratio increased since the polar lipids were tremendously decreased with drought stress. This change of FS/PL reduced membrane fluid, which may diminish DBI-induced fluidity of membrane. However, the unsaturation of polar lipid fatty acids may be a more dominant factor in regulating membrane fluidity than the free sterols.

Veldstra (1944) suggested that changes in membrane permeability to water and solutes could be one of the principal effects of growth substances. Glinka (1971)

found that kinetin treatment decreased permeability to water in carrot tissues and in *Pelargonium* stem tissues, while the ABA increased permeability in the same systems. Also Feng (1973) found that kinetin increased permeability of urea and thiourea in *Allium cepa* cells. Stillwell and Hester (1983) found that kinetin increase water permeability of liposomes composed of several types of phosphatidylcholines including the natural phospholipids and the synthetic phospholipids. It was found that cytokinin was more active in stimulating permeability to urea and erythritol, while auxins, gibberellin, and abscisic acids could stimulate permeability, but only in higher concentrations (Hester, 1984; Stillwell and Hester, 1983). Overall, cytokinins were thought to be a more direct agent in altering membrane permeability (Hester, 1984). Cytokinin stimulates more solutes to penetrate the membrane, therefore decreasing the osmotic potential of the cells and making cells absorb and hold more water. The change of membrane permeability could be attributed to the influence of cytokinin on the protein component of the membrane (Feng, 1973). So far, little has been known about the effect of cytokinin and other growth substances on membrane fluidity, specifically on the polar lipids and free sterols. Whether or not the FSE-induced alteration of membrane lipid composition is engendered by cytokinin is as yet unknown.

Foliage application of FSE could cause an accumulation of cytokinin-like substance in the leaves. The cytokinin could convert the storage compounds to osmotically active compounds (Bewli and Witham, 1976). Also the higher level of cytokinin implies a physiological sink, which could stimulate translocation of osmotically active compounds, like amino acids, carbohydrates, into the leaves

(Mooney and Van Staden, 1986), and finally help plants to hold more water and increase plant drought tolerance.

The FSE could affect cell membrane lipid composition, such as TPLFA and TFS. However, the FSE-induced changes in membrane lipids was not as remarkable as PPC-induced change, but was large than the control ryegrass. The drought tolerance in FSE-treated ryegrass was lower than PPC-treated ryegrass and higher than the control ryegrass. This may imply that the alteration of membrane lipid composition highly related with drought tolerance. However, since the FSE has a complicated composition, further research is needed to determine the effect of each individual plant growth substance on cell membrane lipid composition, and to confirm the role of each plant growth substance impact on drought tolerance.

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## **Chapter 6**

# **Effects of Plant Growth Regulators on Polar Lipid Composition and Salinity Resistance**

### **Introduction**

Salinity stress is frequently a factor reducing turfgrass quality, especially serious in arid areas where frequent and heavy irrigation is combined with high evaporation rates. Physiological drought, nutrient deficiency, and ionic toxicity are most commonly observed in plants exposed to saline environments (Levitt, 1980). Plants growing successfully in saline environments can either exclude the salt from their cells or tolerate the salt by compartmentating the salt in the vacuole (Muller and Santarius, 1978). However, how the plants acquire the salt resistance is still not completely understood. Recently, plant cell membranes have been recognized as stress-sensitive sites within plant cells (Heber and Santarius, 1973). Components of cell membranes, polar lipids and free sterols, can change in their composition and concentration under salinity stress. Erdei et al. (1980) found that polar lipid composition and free sterols of salt-sensitive and salt-tolerant *Plantago* different

responses when they were subjected to NaCl solutions. Muller and Santarius (1978) found in barley leaves (*Hordeum vulgare*. L) seedling that concentration of galactolipids (major chloroplast membrane component) significantly decreased. They thought that sodium chloride accumulated in leaf cells could affect lipid-synthesising enzymes, such as galactosyl transterase and acylase which are attached to the chloroplast envelope. It is suggested that the decrease in galactolipids of membranes is one of the factors causing increased salt resistance in barley which are adapted to extreme salinity. Kuiper (1968) also reported that highest sterol concentration was found in the grape variety which had lowest chloride accumulation. These studies implied that genetic diversity of salinity-resistance existed in plants may be expressed on cell membranes. In other words that salt tolerance could be related to the cell membrane composition (Muller and Santarius, 1978).

Recently, the application of plant growth regulators (PGRs) has become common in turfgrass management. Some growth regulators were found to improve plant salinity resistance (Nabati, 1991), which is very significantly beneficial for turfgrass grown under saline irrigation. However, very little is known about the mechanism of salt-tolerance in turfgrass treated with PGRs. It is hypothesized that PGR-induced salt-tolerance may be related to the influence of PGRs on cell membranes. In this study, the cellular membrane lipid (polar lipid) composition was analyzed in perennial ryegrass treated with NaCl solution and two PGRs: fortified seaweed extract and propiconazole, which had been shown to improve turfgrass salt-resistance (Nabati, 1991). The objectives were to determine cell membrane lipid composition of perennial ryegrass grown under salt and no salt stress conditions, and to ascertain the

influence of FSE and PPC on cell membrane lipid composition and salt resistance.

## Materials and Methods

Perennial ryegrass (*Lolium perenne* L.) seeds were treated with fortified seaweed extract (FSE) and propiconazole (PPC) as described in chapters 4 and 5 (materials and methods sections), and were planted in one liter plastic beakers filled with 1,000 g of sandy soil. When the seedlings were two weeks old, an additional 0.94 ml m<sup>-2</sup> of FSE and 45.8 ul a.i. m<sup>-2</sup> of PPC were sprayed on the foliage with a plastic syringe. When the seedlings were three weeks old, 100 ml of 27.6 mM NaCl solution was applied to the ryegrass every two days for the salinity treatment, whereas 100 ml of potable water was used on the no-salt-treatment ryegrass. The salt treatment continued for 5 weeks until a clear difference in growth and color of ryegrass leaves was observed in the PGR-treated ryegrass and the control (without PGR treatment).

The water stress level in the leaves of each PGR and salt treated ryegrass, as well as in control, was measured just before the harvesting as described in Chapter 4.

The ryegrass leaves were harvested, and 300 mg of them were used for total lipid extraction, polar lipid purification, polar lipid fatty acid analysis, and sterol (free and conjugated) analysis described previously (in chapter 4, materials and methods).

Each treatment was replicated three times and was randomly placed in a greenhouse. Data were analyzed by ANOVA and means were reported with LSD tests.

## Results

Both PPC and FSE -treated ryegrass had significantly higher leaf water potential (LWP) than control (without PGRs treatment) grown on no-salt conditions (Table 6.1). The LWP differences between PGR treated ryegrass and control were even larger when grown under salt-water irrigation (Table 6.1). The PPC-treated ryegrass had significantly higher LWP than the FSE-treated ryegrass under both salt and no-salt treatment (Table 6.1).

The total lipid concentration in FSE and PPC -treated ryegrass, as well as in the control were not significantly different from each other in the no-salt condition (Table 6.2). However, in the salt treated plants, the total lipid concentration decreased in the control ryegrass, whereas the concentration maintained at same level in PPC and FSE -treated ryegrass (Table 6.2).

The PPC-treated ryegrass exhibited a significantly lower level of total polar lipid fatty acids (TPLFA) than that in the control and FSE-treated ryegrass in the no-salt treatment (Table 6.2). However, with the salt treatment, the TPLFA concentration increased in PPC-treated ryegrass and decreased significantly in the control (Table 6.2). The TPLFA concentration in FSE-treated ryegrass was been affected little by salt treatment, and always appeared slightly higher than that in the control under both salt and no-salt treatment (Table 6.2).

Fatty acid composition of polar lipids was changed by the PGR and salt treatments. The PPC-treated ryegrass exhibited a lower proportion of unsaturated

fatty acids ( $C_{18:3}$ ,  $C_{18:2}$ ,  $C_{18:1}$ ) and higher proportion of saturated fatty acids ( $C_{16:0}$ ,  $C_{18:0}$ ) than control under no-salt treatment (table 3), therefore the double bond index (DBI) of polar lipid fatty acids was significantly lower in PPC-treated ryegrass than in the control under the no-salt conditions (Table 6.1). In contrast, under the salt treatment, the PPC-treated ryegrass had a higher proportion of unsaturated fatty acids ( $C_{18:3}$ ) and lower proportion of saturated fatty acids ( $C_{16:0}$ ,  $C_{18:0}$ ) than control, so the DBI significantly increased to 2.06 compared with the DBI (1.37) under no-salt treatment (Table 6.1).

The FSE-treated ryegrass also had a lower DBI than the control under no-salt condition (Table 6.1). This was exhibited by a lower proportion of unsaturated fatty acids ( $C_{18:2}$ ,  $C_{18:1}$ ) and higher proportion of saturated fatty acid ( $C_{16:0}$ ) in FSE-treated ryegrass than that in the control (Table 6.3). However, with the salt stress, an increase of unsaturated fatty acid ( $C_{18:3}$ ,  $C_{18:2}$ ,  $C_{18:1}$ ) and a decrease of saturated fatty acid ( $C_{16:0}$ ) was found in FSE-treated ryegrass (Table 6.3). The DBI of FSE-treated ryegrass increased from 1.48 (no-salt treatment) to 1.74 (salt treatment) (Table 6.1). However, the difference in fatty acid composition between control and FSE-treated ryegrass was not significant after the salt treatment (Table 6.3).

The polar lipid fatty acid composition in the control ryegrass was not significantly changed by salt stress (Table 6.3), neither the DBI was not significantly changed (Table 6.1).

Total free sterol (TFS) concentration and FS/PL ratio in PPC and FSE-treated ryegrass were lower than those in the control under no-salt treatment (Table 6.4 and Table 6.1). The decrease of TFS was mainly due to the decrease in demethyl sterol

(DeMS) level. With salt stress, TFS and FS/PL in FSE-treated ryegrass increased significantly, which was due to the increase in DeMS, whereas the TFS and FS/PL in the control and PPC-treated ryegrass remained unchanged (Table 6.4 and Table 6.1).

Steryl ester (STE) was a major conjugated sterol in ryegrass, and accounted for 72-83% of total conjugated sterols. A higher concentration of STE was found in PPC and FSE-treated ryegrass under no-salt treatment, thereby resulting in a higher level of total conjugated sterol in PPC and FES-treated ryegrass than that in control (Table 6.4). The salt stress caused an increase of STE and total conjugated sterols in the control, whereas all conjugated sterols in PPC and FSE -treated ryegrass remained unchanged in PGR-treated ryegrass (Table 6.4).

The PPC-treated ryegrass contained more Na and Cl than the control or the FSE-treated ryegrass under the no-salt treatment (Table 6.5). All ryegrass grown under salt stress contained more Na and Cl than ryegrass without salt treatment. However, under salt stress, the Na and Cl concentration were significantly higher in the control than that in PPC and FSE-treated ryegrass. K concentration decreased and Ca concentration increased in all ryegrass with salt stress except Ca in PPC-treated ryegrass was affected little.

**Table 6.1. Double bond index, ratio of free sterols to polar lipids and leaf water potential in leaves of perennial ryegrass treated with or without propiconazole, fortified seaweed extract, and salt (NaCl).**

Treatment	DBI	FS/PL	LWP (MPa)
		no salt	
Control	1.65 b	1.45 a	-1.59 c
FSE	1.48 c	0.73 b	-1.50 b
PPC	1.37 c	0.98 b	-1.22 a
		salt stress	
Control	1.72 b	1.75 a	-3.86 f
FSE	1.74 b	1.43 a	-2.68 e
PPC	2.06 a	0.66 b	-2.32 d

Within columns, means followed by a different letters are significantly different based on LSD at  $\alpha = 0.1$ .

DBI = double bond index =  $\Sigma$  ( % of each fatty acid X double bond number in the fatty acid).

FS/PL = ratio of free sterols to polar lipid fatty acids.

LWP = leaf water potential.

PPC = propiconazole.

FSE = fortified seaweed extract.

**Table 6.2. Total lipids and total polar lipid fatty acids concentration in leaves of perennial ryegrass treated with or without propiconazole, fortified seaweed extract, and salt (NaCl).**

Treatment	Total Lipids	TPLFA
	----- mg g <sup>-1</sup> dry wt -----	
	no salt	
Control	54.42 a	5.29 b
FSE	61.62 a	5.99 b
PPC	60.12 a	4.24 c
	salt stress	
Control	40.83 b	4.51 c
FSE	58.65 a	4.92 bc
PPC	62.08 a	7.45 a

Within columns, means followed by a different letters are significantly different based on LSD at  $\alpha = 0.1$ .

TPLFA = total polar lipid fatty acid.

PPC = propiconazole.

FSE = fortified seaweed extract.

**Table 6.3. Polar lipid fatty acid concentration in leaves of perennial ryegrass treated with or without propiconazole, fortified seaweed extract, and salt (NaCl).**

Treatment	C <sub>14:0</sub>	C <sub>14:1</sub>	C <sub>16:0</sub>	C <sub>18:0</sub>	C <sub>18:1</sub>	C <sub>18:2</sub>	C <sub>18:3</sub>
----- % -----							
no salt							
Control	4.97 a	4.03 a	24.26 b	8.18 a	2.26 a	10.37 a	45.90 b
FSE	5.42 a	4.73 a	30.94 ab	8.02 a	0.68 c	7.45 b	42.42 b
PPC	4.19 a	3.05 b	35.95 a	9.19 a	0.67 c	7.11 b	39.88 b
Salt Stress							
Control	3.00 a	3.46 ab	24.70 b	7.51 a	2.15 a	11.43 a	47.94 ab
FSE	3.45 a	3.25 ab	21.95 b	10.36 a	1.42 b	8.94 ab	50.61 a
PPC	3.90 a	2.00 b	17.28 b	4.86 b	1.51 b	9.11 a	61.33 a

Within columns, means followed by a different letters are significantly different based on LSD at  $\alpha = 0.1$ .

PPC = propiconazole.

FSE = fortified seaweed extract.

**Table 6.4. Free sterols and conjugated sterols in leaves of perennial ryegrass treated with or without propiconazole, fortified seaweed extract, and salt (NaCl).**

Treatment	Free Sterols			Conjugated Sterols			
	DiMS	DeMS	Total	SG	ESG	STE	Total
----- mg g <sup>-1</sup> dry wt -----							
no salt							
Control	4.02 a	3.62 a	7.64 a	0.58 ab	0.94 a	2.57 c	3.79 c
FSE	3.17 a	1.24 c	4.41 b	0.59 ab	0.41 b	5.06 b	6.06 ab
PPC	3.15 a	1.02 c	4.17 b	0.18 b	0.19 b	4.98 b	5.35 b
salt stress							
Control	5.37 a	2.53 b	7.90 a	0.67 ab	0.49 ab	6.37 a	7.53 a
FSE	4.48 a	2.50 b	6.98 a	1.14 a	0.58 ab	4.46 b	6.18 ab
PPC	3.20 a	1.72 bc	4.92 b	0.15 b	0.16 b	4.93 b	5.24 b

Within columns, means followed by a different letters are significantly different based on LSD at  $\alpha = 0.1$ .

DiMS = dimethyl sterols.

DeMS = demethyl sterols.

SG = steryl glycosides.

ESG = esterified steryl glycosides.

STE = steryl ester.

PPC = propiconazole.

FSE = fortified seaweed extract.

**Table 6.5. Concentration of K, Ca, Na, and Cl in leaves of perennial ryegrass treated with or without propiconazole, fortified seaweed extract, and salt (NaCl).**

Treatment	K	Ca	Na	Cl
----- % -----				
no salt				
Control	2.40 a	0.65 b	0.07 d	1.26 d
FSE	2.28 a	0.70 b	0.11 d	1.41 d
PPC	2.00 a	0.99 ab	0.84 c	2.93 c
salt stress				
Control	1.40 b	1.70 a	3.00 a	7.52 a
FSE	1.45 b	1.52 a	1.93 b	5.23 b
PPC	1.51 b	1.25 ab	2.12 b	5.52 b

Within columns, means followed by a different letters are significantly different based on LSD at  $\alpha = 0.1$ .

PPC = propiconazole.

FSE = fortified seaweed extract.

## Discussion

Salinity-induced damages in plants has been found in many aspects of morphology, physiology, and biochemistry. Bernstein (1964) classified the effects into three major categories: osmotic, nutritional, and toxic effects. Osmotic dehydration, or so-called physiological drought stress could be immediately caused in plants subjected to saline environment (Krist, 1977; Gabr et al., 1977; Ehrlert, 1960). Unbalanced ion uptake because of ion suppression associated with high Na and Cl concentration could cause K (Giorgi et al., 1967; Osmond, 1968), Mg (Ansari, 1972; Austenfeld, 1974), Ca (Poonia et al., 1972; Matar et al., 1975), and P (Zhukovskaya, 1973) deficiency. Furthermore, direct toxic effect of Na and Cl on ATPase activity (Kuiper, 1984), amino acid metabolism (Shevyakova and Komizerko, 1969) have been reported. Levitt (1980) reviewed the damaging effect of salinity on whole plant, cellular, organella, and molecular levels.

Greenway and Munns (1980) indicated that salt-tolerance is a complex phenomenon which involves morphological, physiological, and biochemical processes. Many adaptive process of plants to salinity, such as ion transfer and compartmentation, and osmotic adjustment were reviewed (Munns and Termaat, 1986; Poljakoff-Mayber, 1982). These adaptive processes may directly or indirectly relate to cell membrane alteration. The cell membranes have been recognized as the most sensitive and important sites to respond to environmental stress (Heber and Santarius, 1973). In other words, plant adaptation or tolerance to environmental

stress could be due to the alteration of membrane responding to the environment (Muller and Santarius, 1973).

Polar lipids and free sterols are major components of cell membrane. The alteration of the polar lipids could directly change membrane fluidity and permeability, while the fluidity and permeability of membrane may be a key trigger to salinity resistance. Erdei et al. (1980) found that phospholipid levels in the roots of salt-tolerant *Plantago* species was lower than that in salt-sensitive *Plantago* species under no salt condition. The phospholipid level increased in the salt-tolerant species, whereas decreased in the salt-sensitive species under salinity solution. This conformed to Ferguson's report (1966) that NaCl treatment increased phospholipid level in salt-tolerant barley. Possibly, phospholipid synthesis in the salt-tolerant species was stimulated by minor salt stress (Erdei et al., 1980).

Elevated phospholipids may relate to increased vesiculation of the plasmalemma and tonoplast (Flowers and Hall, 1973) as well as the increased rough endoplasmic reticulum (ER) (Yeo et al., 1977) in the plants exposed to NaCl solution. The increase of vesiculation of plasmalemma and rough ER may contribute to regulation of ion fluxes under salt stress in order to maintain a favorable ionic composition in plant cells and tissues (Erdei, 1980; Kuiper, 1984). Whereas the decrease of total lipid or phospholipid level may be due to either reduced synthesis or accelerated degradation of the lipid by NaCl (Kuiper, 1984).

In this study, the total lipids and total polar lipid fatty acid (which are mostly phospholipids) concentration significantly increased in PPC-treated ryegrass, whereas they decreased in the control when they were subjected to salt stress (Table 6.2).

These results suggest the PPC-treated ryegrass had a similar response to salt treatment as salt-tolerant species, whereas the control had similar response as the salt-sensitive species to salt stress. The FSE-treated ryegrass exhibited insignificant changes in total lipid and TPLFA concentration under salt stress, which implied that FSE-treated ryegrass was less sensitive to salt than the control, but also less tolerant to salt than PPC-treated ryegrass.

Polar lipid fatty acid composition is important in regulating membrane fluidity and ion permeability, which could directly influence the active and passive ionic flux in plants. Scarpa and de Gier (1971) found that the increasing unsaturation of phospholipid in cell membrane could cause a significant increase in  $K^+$  permeability together with a much smaller increase in  $Na^+$  permeability. This indicated a significant discrimination in permeability between  $Na^+$  and  $K^+$  in the highly unsaturated membranes. Under salt (Na and Cl) stress, the high ratio of Na / K often causes damage to K-required metabolic processes. Therefore the greater unsaturation of cell membranes, which are more permeable to K than to Na, could serve to reduce salt-induced damages to membrane.

In this study,  $Na^+$  concentration in ryegrass was inversely correlated with the membrane polar lipid unsaturation (or DBI) in all treated ryegrass (Table 6.1, 6.5) either under no-salt or salt stress condition, which is consistent with the results of Scarpa and de Gier's findings (1971). The PPC-treated ryegrass had less unsaturated polar lipid fatty acid (low DBI) than the control under no-salt treatment, and exhibited higher  $Na^+$  and slightly lower  $K^+$  concentrations than the control (Table 6.5). The DBI in FSE-treated ryegrass was between that in control and in the PPC-treated

ryegrass, so the Na<sup>+</sup> and K<sup>+</sup> concentration in FSE-treated ryegrass was in the range between that of the control and PPC-treated ryegrass (Table 6.5). Similarly, under salt treatment, the PPC-treated ryegrass had the highest DBI and unsaturation of polar lipids associated with the highest K<sup>+</sup> and lowest Na<sup>+</sup> concentration level. Control ryegrass had the lowest DBI value and the highest Na<sup>+</sup> and lowest K<sup>+</sup> concentration (Table 6.5). Cl<sup>-</sup> concentration changed in a similar manner as the Na<sup>+</sup> by PGRs and salt treatment (Table 6.5). The significantly lower Na<sup>+</sup> and Cl<sup>-</sup> concentration in PPC and FSE -treated ryegrass under salt stress treatment could be very important for salt resistance.

Furthermore, in order to resist or tolerate the salinity-induced physiological drought stress, plants usually decrease their osmotic potential to keep or gain necessary water. The increased unsaturation of polar lipid fatty acids of membrane could enhance the permeability of water and osmotic solutes, such as organic acids and starch, to facilitate osmotic adjustment.

However, these results were different from those refereed by Harzallah-Skhiri et al. (1982) who showed that an increase in C18:2 level together with decrease in C18:3 level occurred in the salt-tolerant plants exposed on salt stress . Harzallah-Skhiri et al. (1982) pointed out the change of fatty acid composition might lessen the membrane fluidity and decrease ion permeability and, therefore, were considered as a part of regulation process of salt resistance in plants. This may be true in the plants under initial adaptive process to mild salt stress. However, in this study, the PPC and FSE-treated ryegrass showed a lower unsaturation of polar lipids under the no-salt treatment and higher unsaturation under the serious salt stress (Table 6.3) to

tolerate more serious salt stress.

Free sterols are effective regulators in membrane stability and ionic permeability (Shinitzky, 1984; Demel and De Kruijff, 1976; Kuiper, 1985). Kuiper (1968) found that the lowest free sterol level was in the roots of the most salt-sensitive varieties of three. The same results had been observed in sugar beet roots (Stuiver et al., 1978). Generally, a high level of free sterols in cell membranes could reduce the fatty acyl chain mobility and fluidity, and reduce the ion passive translocation. But under extreme stress, the more fluid membrane may be necessary for survival.

In this study, the lower levels of total free sterols were found in PPC and FSE-treated ryegrass than in the control under salt treatment (Table 6.4), which indicated that PPC and FSE-treated ryegrass had more fluid membranes than the control under the extreme salt stress. However, the TFS levels in PPC and FSE-treated ryegrass were lower than the control under no-salt treatment (Table 6.4), which played an opposing role that polar lipids played, reduced membrane fluidity. The decrease of total free sterols was mainly attributable to the DeMS which was more sensitive to salt stress and PGRs treatment. The lower level of DeMS in PPC-treated ryegrass may result from the fact that PPC is an inhibitor of sterol demethylation, thereby reducing DeMS synthesis (Kato, 1986; Sisler and Ragsdale, 1984).

This study indicated that alteration of cell membrane composition plays an important role in salt tolerance. There are many factors which could contribute to the changes in membrane fluidity or unsaturation; however, the polar lipid fatty acid unsaturation and their content are most important. The PPC and FSE could improve salt tolerance because that they were able to change the cell membrane lipid

composition under salt stress condition. However, the PPC affected membrane lipid unsaturation more strongly than FSE did, therefore the PPC-treated ryegrass had higher salt tolerance than the FSE-treated ryegrass. Furthermore, how the FSE and PPC affect these alteration of membrane lipid composition requires further research.

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## **Chapter 7**

# **The Influence of Plant Growth Regulators on Turf Quality and Nutrient Element Efficiency**

### **Introduction**

Traditionally, good turfgrass quality is maintained by high fertilization. However, application of huge amounts of fertilizer could result in nitrate and phosphate contamination to ground or surface water. Therefore, reducing fertilization while maintaining turfgrass quality becomes important in an environmentally-concerned community. Plant growth regulators (PGRs) may play an important role in solving this problem. The PGRs are applied in a small amounts which can be directly absorbed by blades and hence are of little risk as an environmental pollutant.

It has been shown that seaweed extract (SE) and triazole chemicals can stimulate plant root growth and development; delay senescence; maintain turfgrass color, and increase plant tolerance to environmental stresses (Schmidt, 1990; Goatley and

Schmidt, 1990a; Crouch, 1990; Fletcher and Hofstra, 1985; Fletcher et al., 1986). These effects may be attributed to the influence of the PGRs on endogenous growth substances.

Recently, it has been demonstrated that SE contains cytokinins or cytokinin-like substances (Bentley-Mowat and Reid, 1968; Hussian and Boney, 1969; Mooney and Van Staden, 1986; Brain et al., 1973; Sanderson and Jameson, 1986; Crouch, 1990), and when SE is applied to plants, the endogenous cytokinin levels increase (Crouch, 1990; Schmidt, 1990, Yan, in Chapter 2). Seaweed extract-treated plants showed the similar response as plants treated with cytokinins (Goatley, 1988; Schmidt, 1990; Crouch, 1990). Other growth substances were also found in SE, which included auxins (Williams et al., 1976; Kingman and Moore, 1982; Sumera and Cajipe, 1981), gibberellin acid (GA)-like substances (Mowat, 1963, Taylor and Wilkinson, 1977) that had impact on stimulating plant growth.

Generally, the triazole chemicals are considered inhibitors of GA biosynthesis in plants, specifically, they inhibit the oxidation of *ent*-kaurene to *ent*-kaurenoic acid (Koller, 1987). The inhibition of GA could increase the concentration of ABA and cytokinin, because these growth substances have a common precursor, since their biosynthesis involved the mevalonic acid pathway (Devlin and Witham, 1983). It is also well documented that N-P-K fertilizer could significantly influence plant endogenous substances. Moorby and Besford (1983) indicated that fertilizer influences plant growth indirectly via its effect on the supply of growth substances which resemble cytokinins, and auxins. Salama and Wareing (1979) thought that varying nutrient conditions affect the endogenous growth substance level which, in

turn, affect the pattern of growth response. Numerous studies reported the effects of nutrition on endogenous growth substance levels, such as GA (Rajagopal and Rao, 1974), ABA (Goldbach et al., 1975), and cytokinins (Salama and Wareing, 1979; Darrall and Wareing, 1981).

Since PGR and fertilizer may have same functions, for example, both of them could influence on endogenous growth substances. Thus it should be possible to reduce fertilization with application of PGR. This study attempted to observe the effects of FSE and PPC on turf quality and nutrient uptake and utilization under different fertilization levels, to ascertain the possibility of reducing fertilization requirement.

## **Materials and Methods**

'Plush' Kentucky bluegrass (*Poa. pratensis* L.) was seeded ( $9.7 \text{ gm}^{-2}$ ) in 4 liter (16 cm diameter) containers filled with sandy soil (pH of 6.3). All containers were placed in a greenhouse under a mist system and were supplied with 3.5 mm water per day. Three levels of 20-20-20 (N-P-K) fertilizer were applied weekly starting when the bluegrass seedlings were two weeks old. The high, medium, and low fertilization levels were  $1.35 \text{ g N m}^{-2}$ ,  $0.67 \text{ g N m}^{-2}$ , and  $0.34 \text{ g N m}^{-2}$  at each application for a total of 10 times. The PGRs: FSE (supplied by Root Inc., New Haven, Connecticut) and PPC (Ciba-Geigy, Greensboro, NC) were sprayed monthly on foliage with a 10 ml syringe starting when the grass seedlings were 25 days old. Each application dosage was  $0.94 \text{ ml m}^{-2}$  for FSE and  $45.8 \text{ ul a.i.m}^{-2}$  for PPC. Density and color were

evaluated weekly on a scale from 1 to 9 with 1 indicating poor density or color and 9 indicating excellent turfgrass density or dark green color. Clippings were harvested monthly before each application of the PGRs, then dried in a forced air oven at 65 C for 24 hours. All clipping samples were combined and analyzed for nutrient element content. Nitrogen concentration was determined by the micro-Kjeldahl method. The other elements (P, K, Ca, Mg, Zn, Mn, Fe) were determined by Inductively Coupled Plasma spectrometry (ICP).

Experimental design was a randomized complete block with four replications. A two factor analysis of variance (ANOVA) and a least significant difference (LSD) test were performed on each data set.

The uptake efficiency is defined as the total nutrient elements absorbed per unit applied fertilizer level (Maranville et, al. 1980), which is equal to the total clipping yield ( $\text{gm}^{-2}$ ) times internal concentration of absorbed element divided by the fertilizer level ( $\text{gm}^{-2}$ ). The utilization efficiency is defined as the total clipping yield ( $\text{g/m}^{-2}$ ) per unit of absorbed element (Maranville et, al. 1980), which is equal to the total clipping yield divided by the total absorbed element in turfgrass ( $\text{gm}^{-2}$ ).

## **Results and Discussion**

### **Effect of Fertilization Levels and PGRs on Turf Quality**

The average density rating of bluegrass was 4.7 at low fertility, 5.8 at medium fertility, and 6.9 at high fertility (Table 7.1). The average color rating of the bluegrass was 4.8 at low fertility, 5.6 at medium fertility, and 6.8 at high fertility (Table 7.2).

Clipping yield of the bluegrass was 79.3 gm<sup>-2</sup> at low fertility, 116.2 gm<sup>-2</sup> at medium fertility, and 188.5 gm<sup>-2</sup> at high fertility (Table 7.3). The improvement of turfgrass quality (color, clipping yield) may be attributed to the effect of the N-P-K fertilizer on endogenous cytokinins. Salama and Wareing (1979) found that endogenous cytokinin can decrease within a few days in all parts of sunflower plants when they were transferred to a nitrogen deficient solution, and potassium and phosphate deficiencies also reduced the cytokinin level. In other words, N-P-K fertilizer could stimulate endogenous cytokinin level; then the enhanced cytokinin would protect the chloroplast from oxidation, stimulate cell division and enlargement.

The bluegrass treated with FSE and PPC always showed higher color rating at any fertilization level than the control (without PGRs treatment) (Table 7.2). Density rating tended to be higher in FSE and PPC -treated bluegrass than control at low and medium fertilization level but statistically not significant (Table 7.1). The clipping yield in FSE and PPC -treated bluegrass was higher than the control bluegrass at low and medium fertilization levels (Table 7.3).

**Table 7.1. Density of Kentucky bluegrass treated with different fertility and plant growth regulators (PGRs).**

PGRs	High Fertility (1.35 g N m <sup>-2</sup> )	Medium Fertility (0.67 g N m <sup>-2</sup> )	Low Fertility (0.34 g N m <sup>-2</sup> )
Control	7.0 a	5.6 a	4.6 a
FSE	6.9 a	5.9 a	4.8 a
PPC	6.7 a	5.8 a	4.7 a
Average	6.9 A	5.8 B	4.7 C

Density rating based on a scale of 9 = excellent density, 1 = poor density.

Within columns, means followed by a different letters are significantly different based on LSD at  $\alpha = 0.1$ .

FSE = fortified seaweed extract.

PPC = propiconazole.

**Table 7.2. Color rating of Kentucky bluegrass treated with different fertility and plant growth regulators (PGRs).**

PGRs	High Fertility (1.35 g N m <sup>-2</sup> )	Medium Fertility (0.67 g N m <sup>-2</sup> )	Low Fertility (0.34 g N m <sup>-2</sup> )
Control	6.5 b	5.3 b	4.6 b
FSE	6.8 ab	5.7 a	4.9 a
PPC	7.1 a	5.7 a	4.9 a
averages	6.8 A	5.6 B	4.8 C

Color rating based on a scale of 9 = green, 1 = yellow.

Within columns, means followed by a different letters are significantly different based on LSD at  $\alpha = 0.1$ .

FSE = fortified seaweed extract.

PPC = propiconazole.

**Table 7.3. Clipping yield (gm<sup>-2</sup>) of Kentucky bluegrass treated with different fertility and plant growth regulators (PGRs).**

PGRs	High Fertility (1.35 g N m <sup>-2</sup> )	Medium Fertility (0.67 g N m <sup>-2</sup> )	Low Fertility (0.34 g N m <sup>-2</sup> )
Control	189.5 a	106.5 b	73.0 b
FSE	187.0 a	122.0 a	77.5 ab
PPC	189.0 a	120.0 a	87.5 a
Average	188.5 A	116.2 B	79.3 C

Within columns, means followed by a different letters are significantly different based on LSD at  $\alpha = 0.1$ .

FSE = fortified seaweed extract.

PPC = propiconazole.

**Table 7.4. Internal Concentration of nutrient elements in Kentucky bluegrass treated with different fertility and plant growth regulators (PGRs).**

PGRs	N	P	K	Ca	Mg	Zn	Mn	Fe
	%					ppm		
High Fertility (1.35 g N m <sup>-2</sup> )								
Control	2.98	0.61	2.80	0.74	0.41	108.75	196.25	869.3
FSE	2.69 *	0.53 *	2.46 *	0.64 *	0.35 *	83.75 *	181.75	1187.0
PPC	2.85	0.57	2.55	0.74	0.40	217.00*	176.00	1690.5
Mean	2.84	0.57	2.60	0.71	0.39	136.5	184.7	1248.9
Medium Fertility (0.67 g N m <sup>-2</sup> )								
Control	2.44	0.49	2.35	0.78	0.38	100.25	208.75	841.5
FSE	2.50	0.45	2.27	0.80	0.39	86.25	188.75	1033.3
PPC	2.35	0.45	2.27	0.81	0.42	99.50	172.0	1025.5
Mean	2.43	0.46	2.30	0.80	0.40	107.2	189.8	966.8
Low Fertility (0.34 g N m <sup>-2</sup> )								
Control	2.17	0.43	2.26	0.89	0.48	89.25	251.25	775.8
FSE	2.15	0.40	2.17	0.82	0.43	89.0	221.25	1506.8
PPC	1.95	0.42	2.11	0.87	0.47	137.25	212.0	1269.0
Mean	2.09	0.41	2.18	0.86	0.46	105.2	194.8	1183.8

Within columns, means with '\*\*' at each fertility level section are significantly different from the control based on LSD at  $\alpha = 0.1$ .

FSE = fortified seaweed extract.

PPC = propiconazole.

### **Effect of Fertilization Level and PGRs on Nutrient Element Uptake**

The concentration of N, P, and K in the bluegrass clippings were enhanced by increased fertilizer supply, while concentrations of Ca and Mg were reduced (Table 7.4). This is not surprising since the applied fertilizer mainly contains N, P, and K. Increased fertilization could stimulate both active and passive uptake of N, P, and K.

The bluegrass treated with FSE had low concentration of N, P, K, Ca, and Mg at the high fertility level. The reason for this is not clear. However it may relate to the redistribution of nutrient elements among different organs. FSE has been reported to enhance root growth when fertility is sufficiently high (Goatley and Schmidt, 1990b). This could cause the root to act as a nutrient sink, and result in more nutrient accumulation in the root than in the leaves.

The PPC-treated bluegrass tended to have higher uptake efficiency for every element but Mn at low and medium fertilization, and had lower uptake efficiency for every element but Zn and Fe at high fertilization compared with control. The FSE-treated bluegrass tended to enhance uptake efficiency for every nutrient element at medium fertilization, enhance uptake efficiency for N, K, Zn and Fe at low fertilization, but reduce uptake efficiency for every element but Fe at high fertilization.

The increased uptake efficiency in the two PGR-treated bluegrass may be due to the influence of the PGRs on endogenous growth substance. It is well known that the cytokinin promotes cell division, differentiation, and leaf expansion, therefore enlarging the nutrient "sink" in the developing leaves, and enhancing nutrient element uptake efficiency (Brock and Kaufman, 1991).

**Table 7.5. Nutrient element uptake efficiency in Kentucky bluegrass treated with different fertility and plant growth regulators (PGRs).**

PGRs	N	P	K	Ca	Mg	Zn	Mn	Fe
	$10^2$							
	High Fertility (1.35 g N m <sup>-2</sup> )							
Control	4.16	0.84	3.90	1.04	0.57	1.51	2.75	12.34
FSE	3.71	0.73*	3.39	0.88	0.48	1.15	2.50	16.44
PPC	3.76	0.75*	3.37	0.97	0.53	2.87 *	2.33	21.40 *
Mean	3.87	0.77	3.55	0.96	0.53	1.84	2.53	16.72
	Medium Fertility (0.67 g N m <sup>-2</sup> )							
Control	3.85	0.77	3.60	1.23	0.59	1.53	3.27	13.12
FSE	4.50	0.82	4.10	1.44	0.71 *	1.56	3.37	19.02
PPC	4.19	0.81	4.04	1.44	0.75 *	1.77	3.08	18.34
Mean	4.18	0.80	3.914	1.37	0.68	1.62	3.24	16.82
	Low Fertility (0.34 g N m <sup>-2</sup> )							
Control	4.62	0.92	4.82	1.89	1.02	1.90	5.35	16.35
FSE	4.86	0.91	4.90	1.86	0.99	1.99	5.05	34.50 *
PPC	4.92	1.04	5.31	2.18	1.20	3.27*	5.26	32.08 *
Mean	4.80	0.96	5.01	1.98	1.07	2.38	5.22	27.64

Within columns, means with '\*\*' at each fertility level section are significantly different with the control based on LSD at  $\alpha = 0.1$ .

FSE: fortified seaweed extract

PPC: propiconazole

## **Effect of Fertilization Levels and PGRs on Nutrient Element Utilization**

The absorption and translocation of nutrient elements into plant tissue requires considerable metabolic energy (Devlin and Witham, 1983). If the absorbed nutrients are not used efficiently for growth and development, instead of being accumulated in cells, the metabolic energy and fertilizer will be wasted. Therefore the evaluation of fertilization efficiency not only involves the uptake efficiency, but also the utilization efficiency.

Generally, plants utilize a certain element more efficiently when it is in limited supply (Marschner, 1983). In this study, the high N, P, and K supplement decreased the utilization efficiency of the N, P, and K, and enhanced the utilization efficiency of Ca and Mg (Table 7.6). Plant metabolism requires a certain balance between each essential nutrient element. Increased N, P, K fertilization stimulated the metabolism, which then would require more Ca and Mg, therefore the utilization efficiency of Ca and Mg increased. The FSE-treated bluegrass tended to show more efficient utilization for every element except Fe at low and high fertilization levels, for P, K, Zn and Mn at medium fertilization. The PPC-treated bluegrass consistently had more efficient utilization for N, P, K, and Mn (Table 7.6) at all levels of fertilization.

These results indicated that the PGRs and N-P-K fertilizer both could improve turfgrass color and clipping yield. The effects of PGRs on turfgrass was clearer at lower fertility levels. The mechanism for this may relate to the concentration of endogenous growth substance. Plant growth is enhanced with the increase of cytokinin level to an optimum point; after the optimum growth, the increased cytokinin has no more stimulatory effect and even could retard growth (Brock and

Kaufman, 1991). As it is well known that both fertilizer and the FSE could stimulate endogenous cytokinin level. The low and medium fertilization supplied cytokinin that may be not insufficient for optimum (or maximum) growth, so additional cytokinin supplied by FSE treatment could improve turf growth. However, at high fertilization level, the cytokinin level stimulated by the fertilizer may be high enough to supply an excellent growth condition, so that additional cytokinin produced by FSE treatment may become unnecessary and superoptimal which could retard growth and metabolism. However, the bluegrass treated with PPC showed the same response to the fertilization as the bluegrass treated with FSE, but there was no direct relationship between PPC treatment and endogenous cytokinin level in the current documents. Probably, PPC treatment affected the growth and nutrient efficiency via the regulation to other growth substance, like GA.

Possibly the use of FSE in P, K, Mg, Zn, and Fe deficient soil would be beneficial since FSE is an efficient PGR to stimulate P, K, Mg, Zn, and Fe uptake. The PPC tended to stimulate uptake of all elements but Mn, so using PPC in Mn deficient soil would not be suitable.

In all cases, the possibility of reducing fertilization by the PGRs application is positive. However, it is worth to note that FSE has cytokinin characteristics and the PPC is a GA inhibitor. Like the growth substances, the concentration of FSE and PPC could highly influence their performance. In this study, only one concentration of the PGRs was used, which may or may not be an optimum concentration. It would be valuable to search for the optimum concentration to improve nutrient efficiency in the future.

**Table 7.6. Nutrient element utilization efficiency in Kentucky bluegrass treated with different fertility and plant growth regulators (PGRs).**

PGRs	N	P	K	Ca	Mg	Zn	Mn	Fe
	10 <sup>3</sup>							
	<u>High Fertility (1.35 g N m<sup>-2</sup>)</u>							
Control	33.6	163.9	35.7	135.1	243.9	9.19	5.09	1.15
FSE	37.2	188.7 *	40.6	156.2*	285.7	11.94	5.50	0.84
PPC	35.1	175.4	39.2	135.1	250.0	4.61*	5.68	0.59 *
Mean	35.3	176.0	38.5	142.1	259.9	8.58	5.42	0.86
	<u>Medium Fertility (0.67 g N m<sup>-2</sup>)</u>							
Control	40.98	204.08	42.55	128.21	263.16	9.97	4.97	1.19
FSE	40.00	222.22	44.05	125.00	256.41	11.59	5.30	0.97
PPC	42.55	222.22	44.05	123.45	238.09	10.05	5.81	0.97
Mean	41.2	216.17	43.55	125.5	252.5	10.53	5.36	1.04
	<u>Low Fertility (0.34 g N m<sup>-2</sup>)</u>							
Control	46.08	232.55	44.25	112.36	208.33	11.20	3.98	1.29
FSE	46.51	250.00	46.08	122.00	232.56	11.23	4.52	0.66
PPC	51.28	238.10	47.39	114.94	212.76	7.29	4.72	0.79
Mean	47.96	240.2	45.91	116.43	217.88	9.91	4.41	0.91

Within columns, means with '\*' at each fertility level section are significantly different from the control based on LSD at  $\alpha = 0.1$ .

FSE: fortified seaweed extract

PPC: propiconazole

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## Chapter 8

### Summary

A series of studies were conducted to examine the physiological and morphological responses of perennial ryegrass (*Lolium perenne* L.) and Kentucky bluegrass (*Poa pratensis* L.) to plant growth regulators (PGRs): seaweed extract and triazole compounds.

Two seaweed extracts: fortified seaweed extract (FSE) and concentrated seaweed extract (CSE), showed cytokinin-like activity in a radish cotyledon expansion bioassay. The concentration 3  $\mu\text{l ml}^{-1}$  of FSE and 0.2  $\mu\text{l ml}^{-1}$  of CSE exhibited similar activity as 2.26  $\mu\text{M}$  and 1.95  $\mu\text{M}$  of synthetic cytokinin 6-Benzylaminopurine (BA) in the bioassay respectively. However, the radish cotyledons growth showed a superoptimal response when the concentrations of FSE and CSE were higher than 3  $\mu\text{m ml}^{-1}$  and 0.2  $\mu\text{m ml}^{-1}$  respectively. Furthermore, the endogenous cytokinin activity in FSE and CSE-treated ryegrass was higher than that in the control (without PGR treatment).

Triazole compounds, propiconazole (PPC) and triadimefon (TRF), inhibited radish cotyledon expansion. This inhibition became increasingly serious as the concentration of PPC and TRF increased. The endogenous cytokinin activity in PPC

and TRF-treated ryegrass was not significantly different from the control ryegrass.

Ryegrass treated with PPC and FSE had higher tolerance to drought and saline stresses than the control ryegrass. The leaf water potential (LWP) in PPC and FSE-treated ryegrass was consistently higher than the control ryegrass with or without drought or saline stress. Furthermore, reduced concentration of Na and Cl were found in PPC and FSE-treated ryegrass under saline stress, which could reduce the salt-induced damage of plant cells.

Drought and saline stresses caused total lipids (TL) and total polar lipid fatty acid (TPLFA) concentrations to decrease in the control ryegrass. However, the TL in PPC-treated ryegrass was higher than in control under no-stress conditions, and was not affected by drought or saline stresses. The concentration of TPLFA decreased in PPC-treated ryegrass under no-stress conditions; however, it was not changed by drought stress, and increased with saline stress.

The proportion of each fatty acid of polar lipids in control ryegrass was not influenced significantly by drought or saline stress. However, the percentage of unsaturated fatty acids ( $C_{18:3}$ ,  $C_{18:2}$ ,  $C_{18:1}$ , or  $C_{14:1}$ ) was lower in PPC-treated ryegrass than in control under no-stress condition, but was higher in PPC-treated ryegrass than in the control under drought or saline stress conditions. The saturated fatty acids ( $C_{14:0}$ ,  $C_{16:0}$ ) of the polar lipids were changed inversely with the unsaturated fatty acids by the PPC and stress treatment. These changes of polar lipid composition could be expressed by the double bond index (DBI). Thus DBI in PPC-treated ryegrass remarkably increased after either drought or saline stress.

The concentration of total free sterols (TFS) and total conjugated sterols (TCS)

in PPC-treated ryegrass were not affected by either drought or saline stress. However, demethyl sterol (DeMS) concentration in PPC-treated ryegrass was lower and dimethyl sterol (DiMS) was higher than in the control without stress influences, which could be attributed to the demethylation inhibition characteristic of PPC. The steryl ester, a major conjugated sterol in ryegrass, accumulated more in PPC-treated ryegrass than in the control, and had was not affected by drought or saline stress.

Polar lipid composition and sterol concentration are sensitive to environmental changes. Thus the different results in lipids and sterols from the drought stress in the winter (Dec. 1990) and from saline stress in the summer (July 1991) were possibly due to the seasonal influences (temperature, day length, etc.). For example, in control ryegrass, concentration of TFS was higher than the concentration of TCS in the summer, whereas the TFS was lower than the TCS in the winter. Furthermore, the lipid composition in FSE-treated ryegrass changed with the different season. The TFS concentration in FSE-treated ryegrass declined through drought stress (in winter), but increased with saline stress (in summer). Concentration of TL and TPLFA in FSE-treated ryegrass was not different from the control in summer, and was not affected by saline stress. However, in the winter, TPLFA concentration in FSE-treated ryegrass was lower than in the control and decreased by drought stress.

The DBI in FSE-treated ryegrass increased through drought or saline stress, although this increase was not as large as in PPC-treated ryegrass. This study showed that the change of DBI in ryegrass from non-stressed to stress conditions was closely related with ryegrass tolerance to drought and saline stresses. The larger the change in DBI, the higher the tolerance of ryegrass to stresses. The alteration of polar lipid

composition and sterol concentration indicates the change of membrane fluidity and permeability. The PGR-induced drought and saline tolerance could highly relate to the membrane adaptation.

It was found that PPC and FSE application improved green color, increased clipping yield, and tended to enhance uptake efficiency of most essential nutrient elements and utilization efficiency of some major nutrient elements (N, P, K, Ca, Mg) in Kentucky bluegrass. These PGR-induced promotions were more apparent at lower fertilization than at higher fertilization levels. This relationship indicated a possibility of reducing fertilization requirements by PGR application.

## Vita

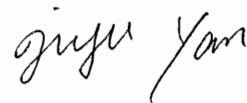
The author was born in Shingtai, Herbei, the people's Republic of China on Octoter, 29, 1960. She attended public schools and graduated from The Second High School of Luan, Anahui in June 1978.

She entered Nanjing Forestry University as a forestry major in August, 1978 and received the Bachelor of Science degree in forestry in June 1982. In August, 1982, She began graduate studies in bamboo ecology and physiology at Nanjing Forestry University and received the Master of Science degree in forestry in June 1985.

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A handwritten signature in cursive script, appearing to read "Jiyu Yan".