EFFECTS OF IMMUNOSUPPRESSANTS ON PLANT GROWTH AND DEVELOPMENT

by

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INTRODUCTION

Wheat is the most versatile among cereal grains in terms of its diversified products. However, wheat proteins do not contain the balance of essential amino acids required by humans and monogastric animals. There are two basic ways to overcome the deficiencies. Supplementation of the limiting amino acids is the easier way of balancing the nutritional status of wheat products, but that generally presumes some processing. Increasing the protein content and/or altering the protein composition of wheat grain genetically is ultimately more economical and offers more potential for improved and new products.

The process of incorporating desired characteristics through varietal hybridization is limited by genetic variability present within the species. An example of this limitation is present in wheat where protein content variations exist but the high lysine character is not available. Wide crosses between genera and species might overcome this limitation if "lysine" genes can be obtained from distant relatives of wheat. Wide crosses are also valuable in wheat improvement programs where transfers of other advantageous characteristics -- disease resistance, seed type, functional properties, etc. -- from related species and genera are desired. Wheat's relationship to other cereals is shown in Figure 1. The intergeneric hybrid triticale, and many new wheat varieties are examples of successful wide crosses.

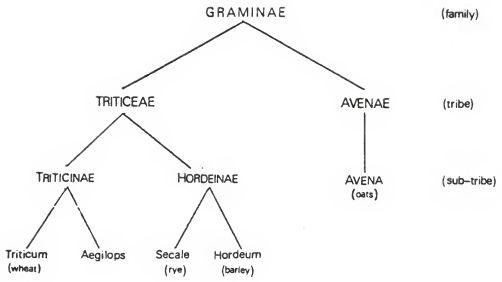

Figure 1. Taxonomic relationship among Gramineae (Bell.1965)

The presence of high lysine genes in barley and their absence in other Triticeae makes barley the only possible donor of high lysine genes for wheat. The success of triticale, the intergeneric cross between rye and wheat,has not been achieved for crosses between barley and wheat. Specific mechanisms to explain the problems in wheat x barley crosses are unknown. The general mechanisms are cross incompatibility and incongruity (Hogenboom, 1975).

It has been hypothesized that crossability barriers are similar to the immunochemical mechanisms in animals. Bates and Deyoe (1973) applied the term stereospecific inhibition reaction (SIR) to the crossability barriers in plants. Bates et al. (1975) further suggested the use of chloramphenicol (CAP), acriflavine, salicylic acid, gentisic acid, and epsilon amino-caproic acid (EACA) to overcome crossbility barriers and reported varying degrees of success in the use of these chemicals to support the theory of stereospecific inhibition reactions.

This research was undertaken to study the effects of the chemicals CAP, acriflavine, salicylic acid, gentisic acid, and EACA on the growth, development and reproductive stages of wheat and barley. Different methods of introduction of the chemicals to the plant -- hydroponics, injection, foliar sprays with and without a carrier -- were also compared.

REVIEW OF LITERATURE

Acriflavine

Acriflavine was the first of the acridine group of dyes to be studied and was initially introduced as a disinfectant. Like most of the acridines it has both bacteriocidal and bacteriostatic effects on microorganisms (Esplin, 1974).

Acriflavine is also known for its mutagenic activity and has been a useful tool in studying extrachromosomal inheritance (Arlett, 1957 and Avers, 1965). In yeast it induced cytoplasmic and respiration mutants and inhibited growth as reported by Tanaka (1971), Asano (1972), and Takahasi (1972). The latter found that there were alterations of mitotic recombinants and production of a number of chromosomal aberrants. Boronin and Sadounikova (1972) stated that mutation was due to the elimination of extrachromosomal inheritance factors. Morita et al. (1972) observed that acriflavine induced respiration mutants only in daughter cells of budding yeasts.

Borisova et al. (1973), Surovaya and Trubitsin (1972), and Potapov et al. (1973) studied the rearrangement of t-RNA due to the binding with acriflavine. Kou (1969) reported that growth of rice and maize coleoptile sections was inhibited by acriflavine and indicated that the inhibition was due to the formation of the DNA-dye complex.

Ashri and Levy (1974) found that acriflavine reduced peanut ovary survival when treated at an early developmental stage. In 1977, in collaboration with Offenbach and Cahaner, they reported the production of aneuploid mutants from the earlier reported treated but

normal peanut plants.

Chloramphenicol (CAP)

Chloramphenicol is an antibiotic produced by Streptomyces venezuelae first isolated in 1947. Its effectiveness against a wide variety of bacteria, viruses, and rickettsias was reported by Ehrlick et al. (1948).

Brock (1961) noted that at low concentration CAP inhibited the growth of animal and plant cells.

Brock (1961) generally attributed the protein synthesis inhibition of CAP to the non-incorporation of amino acids into protein. A number of particular modes of action of CAP, resulting in protein synthesis inhibition, was given by Weinstein (1970): it binds on the 50-s ribosome to prevent binding of m-RNA to ribosomes in microorganisms; suppresses the activity of peptidyl transferase; inhibits conversion of polyribosomes to single ribosomes; and decreases the content of adenosinetriphosphate (ATP). These inhibitory effects were also reported by other researchers in bacteria (Coper, 1974; Coutsogeorgopoulos, 1971 and 1972; Garret and Heman, 1973; Lembach and Buchanan, 1970; Neumann and Partheer, 1973; Nierhaus and Nierhaus, 1973; and Pestka, 1970). Margulies (1962) attributed the inhibitory action of CAP on the development of photosynthesis and chlorophyll formation in pea leaves to its inhibitory effect on protein synthesis in bacteria.

Amino acid incorporation studies in plants treated with CAP agreed with Brock's bacterial data. Bamji and Jagendorf (1966) reported CAP inhibited incorporation of radioactive labelled amino

acids into protein in wheat leaves. In carrot root, Ivanov et al. (1974) found that glysine ^{14}C incorporation was inhibited strongly in the mitochondrial fraction. Inhibited ^{14}leucine incorporation was reported in corn endosperm (Wilson, 1966) and in peas (Nawa and Asahi, 1973).

Jalali and Suryanarayana (1970) observed depressed root exudation of amino acids (but with threonine and asparagine slightly increased) in wheat after foliar treatment of CAP. Alteration of free amino acid concentrations in awned and hooded barley seedlings showed a differential response of these two genotypes to CAP (Sarkissian et al., 1962). Margulies (1966) found that treated chloroplasts contained proportion of one or more proteins than untreated ones.

Chloramphenicol had an inhibitory effect on protein synthesis of 70 s ribosomes of chloroplasts (Sawhney and Naik, 1973; Detchon and Possingham, 1975). It inhibited chloroplast development by altering the properties of chloroplast membranes (Jennings and Ohad, 1972, 1973; Perl, 1972; and Simola, 1973), by inhibiting photosynthesis (Ochai-Yanagi and Matsuka, 1973; and Thinh, 1973), and by decreasing chlorophyll formation (Iordanov and Zeinalov, 1973; Udvardy and Farkas, 1973; and Vichanka et al., 1973). Singh et al. (1973) reported chlorophyll synthesis was inhibited by CAP in isolated cotelydon of watermelon. Nucleic acids and particulate proteins were similarly affected. In barley leaves, chlorophyll accumulation was decreased and growth was inhibited after addition of CAP to the nutrient solution (Shlyk and Kostyuk, 1972, 1973). Shlyk and Averina (1973) found that the decrease in chlorophyll accumulation was counteracted by kinetin.

Other plant responses were associated with the protein plant synthesis by chloramphenicol. CAP lowered water absorption in peas (Nizna, 1976); decreased uptake of salt in corn root (Ivanov et al., 1973); inhibited coleoptile elongation in wheat, oats and barley (Schlender et al., 1972); inhibited root and seedling growth of lettuce (Blaydes, 1970 and le Deunff, 1973); and inhibited callus initiation in potato tissue culture (Austis and Northcote, 1973).

Yoshida et al. (1972) observed chromosome fragmentation and reduced chromosome number in CAP-treated barley root meristematic cells. A complete reduction in chromosome number in barley root from 2n to n was also then reported by Yoshida and Yamaguchi (1973).

Epsilon Aminocaproic Acid (EACA)

Epsilon aminocaproic acid (EACA) is a structural analogue of lysine. It is a known proteolytic enzyme inhibitor (Kaverin, 1967; Back and Steger, 1968; Gillette, 1970; and Troboli, 1970) and an immunosuppressive compound (Gillette et al., 1963; Aversuald and Doleschel, 1966; and Dragomiersau et al., 1973).

In human and laboratory test animals, EACA was reported to be easily absorbed following oral administration and intravenous injection (Nilsson, 1960; McNicol, 1962; Rezakovic, 1966; and Wyrzkiewicz, 1973). Nilsson (1960) reported that EACA was rapidly excreted in the urine. Evered and Hargreaves (1973) reported that the efficient absorption of EACA did not interfere with the uptake of essential amino acid. Hardgrave and Evered (1973) indicated no transamination of EACA was observed.

High concentration of EACA inhibited cell growth without any differences in cellular morphology in cell culture studied by Lisnell and Mellgren (1963). Fertility test with rats showed EACA had no effect in treated female rats both in terms of litter number and size; but reduced the fertility of male rats. There was a twenty percent reduction in litter number (Gunilla and Grant, 1966 and Howarta et al., 1970).

Naumova (1969) mentioned EACA was the first stage product of epsiloncaprolactan hydrolysis in Pseudomonas alacunhae and Bacterium agile.

EACA is one of the immunosuppressants suggested by Bates et al. (1975) to counteract the crossability barriers in cereal wide crosses through the stereospecific inhibition reaction (SIR) theory. Bates (1976) reported the effectiveness of EACA (treatment of the female parents) in controlling the crossability barriers in barley, wheat, and rye crosses. Bates et al. (1977) further reported the enhancement of seed set and embryo development in durum wheat and rye crosses by EACA through the foliar spray treatment. They stated, however, that the foliar spray did not completely overcome the crossability barriers between durum wheat and rye in the case of Boobey "S" S-5 and Tildillo "S" S-12. Taira and Larner (1977) found that three days before to seven days after pollination treatment of EACA, alone or with lysine, significantly increased the development of hybrid embryos in durum wheat and rye crosses. They also reported that the enhancement of embryo development depended on the temperature regimes of 17°C day - 15°C night and 19°C day - 15°C night.

Baker et al. (1975) also reported the advantageous use of EACA in the interspecific cross in the genus Vigna. They found the optimum effective concentration of EACA to be 265 ppm in the cross between mung bean and rice bean.

Salicylic Acid and Gentisic Acid

Salicylic and gentisic acids belong to the secondary plant products, derived from acetate malonate pathway and from shikimic acid (Geisman and Crout, 1969; Ibrahim et al., 1962; Pridham, 1965; and Ribereau-Gayon, 1975).

Leopold and Kriedemann (1975) assessed the secondary plant products, such as the phenolics including the above-mentioned acids, to be inhibitors in the plant. The inhibitory and toxic effects of these compounds on the seedling growth were reported by Varga and Koves (1959), Guenzi and McCalla (1966), Gesto et al. (1967), Wang et al. (1967), and Demos et al. (1975). Although they are considered as inhibitors, they have also been reported to have a stimulatory effect on germination and growth of plants. (Van Sumare et al., 1957; Newman, 1959; Knypl, 1964; and Vieitez et al. 1967).

The research groups of Gesto and Vieitez found that salicylic acid isolated from Castanea sativa and Quercus rubur inhibited Avena coleoptile growth at 50 ppm. With increased concentration, inhibition became more intense and inhibition was completed with coleoptile deaths. Gentisic acid isolated from Quercus rubur had stimulatory effect on coleoptile growth from a low concentration of 10 ppm to a maximum 100 ppm. At higher concentrations stimulation decreased; it became toxic at 150 ppm.

Some phenolics have been associated with indoleacetic acid (IAA) in plants (Hare, 1964). Vieitez et al. (1967) reported that at inhibitory concentrations salicylic acid neutralized the stimulatory effect of IAA on coleoptile growth resulting to the death of coleoptiles. Gesto et al. (1967) found that in combination with IAA, gentisic acid had an additive stimulation of growth but at toxic concentrations the result was parallel with that of the salicylic acid-IAA mixture. In contrast to the aforementioned results, Basu (1969, 1970) reported that salicylic and gentisic acids synergistically promoted the rooting of cuttings with IAA, indolebutyric acid (IBA) and naphthalene acetic acid (NAA). Roy et al. (1972) suggested that the differences in the capacity of cuttings to regenerate was partly due to the differences in the occurrence of phenolic compounds. Salicylic is present in most hard-to-root cuttings.

Kefeli and Kadyrov (1971) hypothesized that the natural inhibitors can penetrate cell membranes and may circulate within the plant. Glass and Bohm (1971) found that simple phenols entered the root of barley by diffusion and were transported actively. Karanov (1967) reported that salicylic acid regulated aging in radish leaves by decreasing the destruction of chlorophyll. Glass (1973) and Demos et al. (1975) stated that salicylic and gentisic acids inhibited phosphate uptake. The former attributed this inhibitory action to the alteration of the membrane properties of the root. These works support Pridham (1965) and Kefeli and Kadyrov (1971): that the toxic and inhibitory effects of phenolic inhibitors are associated with the chemical interference with membrane function, oxidative

phosphorylation, nucleic acid and protein synthesis, which inhibit the processes of growth. Phenolics inhibit stem elongation more actively than other plant growth processes.

MATERIALS AND METHODS

The test plants used were: diploid barley, Hordeum vulgare L. (cv. CM-67, Promesa, Porvenir); tetraploid wheat, Triticum turgidum L. var. durum (cv. Cocorit 71); and hexaploid wheat, Triticum aestivum L. var. aestivum (cv. Tobari).

The chemicals studied were: salicylic acid (2-hydroxybenzoic acid); gentisic acid (2,5-dihydroxybenzoic acid); ε-aminocaproic acid (6-aminohexanoic acid); chloramphenicol acetamide 2,2-dichloro-N-2 hydroxy-1 (hydroxymethyl)-2-(4-nitrophenyl) ethyl; and acriflavine (acridium 2,6-diamino-10-methylchloride mixed 3,6 acridine diamine).

The chemicals were introduced into the plant system: through the roots in hydroponic experiments, into the hollow space of the leaf sheath surrounding the developing spike through injections, and into the leaves through foliar sprays with and without a surfactant carrier (Tergitol S-15, 0.05%).

Hydroponic Experiments

Chemical concentrations ranged from 0 to 1,000 ppm. as tabulated below:

	Concentration (ppm.)				
Chemical	0	1	10	100	1000
Salicylic acid	x	x	x	x	x
Gentisic acid	x	x	x	x	
EACA	x	x	x	x	x
CAP	x		x	x	x
Acriflavine	x		x	x	x

Test plant seeds were germinated in vermiculite. Four-day-old seedlings were then transplanted into two-liter hydroponic pots in which each of the chemical concentrations was added to full strength Hoagland nutrient solution. Each pot supported six seedlings and was continuously aerated. Plants were grown to maturity under the growth chamber conditions of: 14 hours of 42,000 lux illumination, 10 hours of darkness, 26.6°C day and 15.5°C night temperatures, and 42% relative humidity. Experimental solutions were changed every two weeks. In between the solution change pH was adjusted and 2 ml of iron (6% $Fe\ SO_4 \cdot 7H_2O$ - 4% tartrate) was added.

Some plant characteristics, widely used in agricultural research were considered. They were tillering capacity, date of flowering or heading time, plant height and internode measurements where:

a) tillering capacity was recorded as the number of tillers produced;

b) heading time or date of flowering was taken as days after transplanting until the awns emerged from the flag leaf sheath;

c) plant height (in centimeters) at four and six weeks after transplanting was measured from the base of the plant to the extension of the leaves, at maturity was measured from the base of the plant to the spike less awns;

d) internode length (in centimeters) was measured between nodes at the time periods specified in (c). Internodes were numbered from the base of the plant, number 1 was the basal internode. The peduncle was not included in the analysis.

Chromosomal behaviours of somatic and germ cells were also investigated. Germination and chlorophyll mutation tests were run on harvested seeds. Leaf samples were collected for chemical uptake analyses.

Two brief hydroponic experiments were conducted to test the ability of lysine and gibberellic acid (GA_3) to reverse EACA effect. One was carried out in a supported hydroponic system where the following EACA-lysine concentration (ppm) combinations with and without GA_3 were used: 0-0 (control), 100-0, 500-0, 1000-0, 0-240, 1000-240, and 1000-240.* Five seedlings of each species were placed in each EACA-lysine treatment in five replications. The barley and wheat seedlings were individually transplanted into pots containing vermiculite and were irrigated every other day with nutrient solution plus the EACA-lysine concentrations. The plants were irrigated with double distilled water on days when they did not receive nutrient solution. The plants were kept in the growth chamber. GA_3 (10 ppm) was added after four weeks to two replicates of the treatment combinations.

The other brief hydroponic experiment was carried out in straight unsupported hydroponics. Eight EACA-lysine treatment combinations were studied, including an EACA-lysine equimolar concentration treatment. The concentrations (ppm) were 0-0 (control) 100-0, 500-0, 1000-0, 0-559, 500-559 (equimolar), 1000-559, and 1000-559.* Two seedlings of each species were transplanted into each treatment

*Lysine was added a week after the 1000 EACA treatment and thereafter added together.

combination which were replicated twice. This experiment followed the procedure of the main hydroponic experiment with respect to the conditions under which the plants were grown. After four weeks of growth, GA_3 (100 ppm of potassium gibberellate) was added to one of the replicates.

Plant height differences were recorded before GA_3 addition and two weeks after GA_3 addition in each brief hydroponic experiment.

Injection and Spray Experiments

Experiments on the other methods of chemical introduction through injection and foliar sprays were done in the greenhouse. Seedlings from germinated test plant seeds were transplanted in jiffy pots and were kept in the growth chamber for two weeks. They were then potted in soil (3:1:1 mixture of clay soil, peat moss, and sand) in the greenhouse. Three plants of each genotype were assigned for each method of introduction: injection, aqueous spray, aqueous spray with carrier. Six control plants of each test species were included. Half were sprayed with water plus carrier to serve as immediate controls for the aqueous spray with carrier and the other half left untreated as overall controls.

Concentration of 1,000 ppm was used for each of the chemicals investigated. Chemical introductions were made from the booting stage until pollen shedding (approximately 14 treatment days).

Chemicals were in aqueous solution. They were injected, using hypodermic needle, to the plants at the base of the flag leaf and next lower internode immediately below the developing spike. A daily dose of 1.0-1.5 ml was given to the plant. Injection was

stopped as soon as the solution oozed out of the flag leaf or internode. Foliar spray treatments were accomplished by spraying the canopy completely. Spraying was stopped when leaves were fully wetted with the solution. Quart-size hand sprayers were used for the spray treatments.

Embryos were collected for chromosome behaviour. Number and weight of seeds per spike were recorded. Similar germination and chlorophyll mutation tests were carried out on the harvested seeds.

Extraction and Analysis

Leaf samples from the salicylic and gentisic hydroponic experiments were oven dried at 21°C. They were then ground using a micro Wiley mill. Twenty ml of 75% ethanol was added to five grams of ground leaf samples and the suspension was let stand for 24 hours. The samples were centrifuged at 12,000 x g for 20 minutes. The supernatant was analyzed by high pressure liquid chromatography (HPLC) with the following: sample size = 30 l, flow rate = 1.5 ml, chart rate = 0.5 cm/minute, solvent = 5% isopropyl alcohol = CH_2Cl_2, and att. of 04.

Three grams (fresh weight) of frozen leaf samples from EACA hydroponic experiment were homogenized in medium containing 10 ml of 3% sulfosalicylic acid and 1 ml of diluted norleucine (0.065 mg/ml) with mortar and pestle. The extracted material was held at 2°C overnight and then centrifuged at 12,000 x g and 2°C for 20 minutes. Twenty ml of ethyl-ether was mixed with the centrifuged supernatant in a separatory funnel. The aqueous layer was removed from the separatory funnel and concentrated in a rotary evaporator. Five ml of

dilute citrate buffer (pH 2.22) was added to the concentrated sample, which was then ultra-filtered and analyzed for EACA, lysine histidine and arginine. The analysis was performed with an automatic amino acid analyzer.

Analyses of variance were performed on all plant responses and least significant difference (LSD) was used to compare significant treatment means at 5% probability level.

RESULTS AND DISCUSSION

The potential advantage of animal immunosuppresants in cereal crop improvement has been suggested through the stereospecific inhibition reaction (SIR) theory. The primary objective of immunosuppressant use is to overcome the crossability barrier(s) in cereal wide crosses, which is one of many potential SIR-controlled phenomena in plant development.

Hydroponic Experiments

The chemicals studied--salicylic acid, gentisic acid, chloramphenicol and acriflavine--are considered plant inhibitors. EACA is a known enzyme inhibitor in animals. The chemicals decreased plant growth and germination percentages of T_1 (first treated generation) seed and caused death of barley and wheats plants in hydroponics. Somatic cells (root tips and embryos) and germ cells (pollen mother cells) showed no chromosomal abnormalities that can be attributed to effect of the different chemicals or levels of chemicals (K. A. Mujeeb, personal communication). Among the different test plant materials, the hexaploid wheats were the least affected by the chemicals.

Salicylic and Gentisic Acids

Salicylic acid showed toxic effects resulting in the death of barley and wheat plants: at early seedling stage at 1000 ppm concentrations and before the reproductive stage at 100 ppm. The 100 ppm concentration, which was toxic in the case of salicylic acid, was not toxic in the case of gentisic acid; however, it was inhibitory.

The results are in parallel to the findings on Avena coleoptile growth experiments of Vieitez et al. (1967) and Gesto et al. (1967). Both groups reported the toxic effect (death of coleoptiles) of salicylic acid at 80 μg/ml. The latter workers showed gentisic acid to be a growth inhibitor at higher concentrations (100 μg/ml). This toxicity is shared by another benzoic acid derivative, p-hydroxybenzoic acid, in sugar cane cutting growth (Wang et al.,1967). The observations and measurements on test plants that survived the different salicylic acid and gentisic acid regimes are discussed below.

The effect of salicylic acid on height of barley and wheat plants is shown in Table 1. Height of barley (CM-67) and tetraploid wheat (Cocorit 71) were significantly increased at 1 ppm but decreased at 10 ppm compared to their controls. The same trend, though insignificant, was also observed on the hexaploid wheat (Tobari).

Increased and decreased plant heights were also observed in gentisic acid treated plants as shown in Table 2. CM-67 plants at the different concentrations of gentisic acid were taller than their controls; the optimum increase was at 10 ppm. In contrast to the barleys, the Cocorit 71 plants treated with gentisic acid were shorter than the controls and significant decreases were noted at 1 ppm and 100 ppm. Almost uniform heights were observed in the hexaploid wheats. Only those at 10 ppm exhibited a different height expression--they were taller than the rest of the treatments.

The effect of phenolic inhibitors depends on the concentrations as well as plant species (Guenzi and McCalla, 1966 and Wang et al., 1967). Gesto et al. (1967) and Vieitez et al. (1967) in their

Table 1. Effect of salicylic acid on plant height (cm), at maturity, of barley tetraploid and hexaploid wheats grown in hydroponics

	Concentration (ppm)		
	0 (Control)	1	10
H. vulgare cv. CM-67 (2n=14)	56.2a*	64.0b	43.0c
T. turgidum cv. Cocorit 71 (2n=4x=28)	58.7a	64.2b	50.3c
T. aestivum cv. Tobari (2n=6x=42)	63.3a	67.0a	64.0a

*Numbers followed by the same letter are not significantly different at 5% probability.

Table 2. Effect of gentisic acid on plant height (cm) at maturity of barley tetraploid and hexaploid wheats grown in hydroponics

	Concentration (ppm)			
	0 (Control)	1	10	100
H. vulgare cv. CM-67 (2n=14)	58.5a*	66.4b	68.6b	62.5a
T. turgidum cv. Cocorit 71 (2n=4x=28)	68.2a	60.6b	67.2a	63.8b
T. aestivum cv. Tobari (2n=6x=42)	74.0a	73.9a	77.0b	72.0a

*Numbers followed by the same letter are not significantly different at 5% probability.

coleoptile growth experiment have shown that at lower concentrations salicylic acid was an ineffective growth inhibitor while gentisic acid was stimulatory. Similarly, Van Sumare et al. (1973) indicated that phenolic germination inhibitors have stimulatory effect on the process of germination.

Plant height is associated with the length of the internodes when number of nodes and internodes are constant. The results in Tables 3 and 4 agree with reports that taller (or shorter) wheats have longer (or shorter) internodes (Johnson, 1954) and that reduction in height is primarily due to shortening to internodes (Merkle and Atkins, 1964). Short salicylic-treated barley and wheat plants were observed to have shorter internode lengths. Gentisic acid concentrations that increased plant height increased the internode length patterns in hexaploid wheat and barley; those that decreased height (at 1 and 100 ppms) of the tetraploid wheats shortened the internode lengths. The significant effects, however, of gentisic acid and salicylic acid seemed to be at random rather than on particular internodes.

Tillering was observed to be continuous, i.e., it occurred at all growth stages, in both salicylic and gentisic acids—grown plants. Tillering capacity did not differ significantly among test plants. In all cases, a number of young tillers senesced and died on treated as well as on untreated plants.

Concentrations of 10 ppm of salicylic and 100 ppm of gentisic acid delayed the onset of flowering of all species. Flowering of hexaploid wheat was also delayed, but 4x Cocorit 71 flowering was

Table 3. Effect of salicylic acid on internode length (cm), at maturity, of barley, tetraploid and hexaploid wheats grown in hydroponics

Concentration (ppm)	*H. vulgare* cv. CM-67 (2n=14)				*T. turgidum* cv. Cocorit 71 (2n=4x=28)				*T. aestivum* cv. Tobari (2n=6x=48)			
	Internode*				Internode				Internode			
	1	2	3	4	1	2	3	4	1	2	3	4
0 (Control)	4.6a**	7.1a	10.5a	8.5a	4.2a	5.5a	7.0a	9.6a	3.0a	5.9a	8.4a	13.3a
1	4.0a	6.5a	9.2b	10.0a	2.3a	4.9a	7.3a	9.5a	4.1a	5.8a	8.2a	14.3a
10	3.7a	4.3a	6.3c	8.9a	2.5a	4.4a	6.9a	9.4a	3.4a	5.0b	8.3a	13.8a

*Internodes are numbered from the base of the plant upward, number 1 being the basal internode.

**Numbers followed by the same letter are not significantly different at 5% probability level.

Table 4. Effect of gentisic acid on internode length (cm), at maturity of barley, tetraploid and hexaploid wheats grown in hydroponics

Concentration (ppm)	*H. vulgare* cv. CM-67 (2n=14)					*T. turgidum* cv. Cocorit 71 (2n=4x=28)				*T. aestivum* cv. Tobari (2n=6x=48)			
	Internode*					Internode				Internode			
	1	2	3	4	5	1	2	3	4	1	2	3	4
0 (Control)	3.3a**	5.2a	6.2a	6.8a	8.1a	3.0a	5.7a	8.6a	13.5b	4.0c	5.6a	8.6a	14.1a
1	2.8a	7.9a	9.6a	8.0a	9.6a	2.4a	4.4a	6.5a	11.9b	2.5b	5.5a	9.4a	15.1a
10	3.1a	6.5a	6.6a	8.7a	8.8a	2.5a	4.9a	7.3a	9.9a	1.0a	4.8a	8.8a	15.0a

*Internodes are numbered from the base of the plant upward, number 1 is the basal internode.

**Numbers followed by the same letter are not significantly different at 5% probability.

accelerated by eight days with salicylic acid.

No chromosomal abnormalities were observed from the somatic and germ cells of plants treated with salicylic acids.[1] Chromosomes and satellites were distinct (K. A. Mujeeb, personal communication).

Both acids had no effect on the subsequent T_2 seed germination rate nor were chlorophyll mutants obtained.

Chemical analysis of the leaf samples by high pressured liquid chromatography (HPLC) failed to indicate the presence of salicylic acid and gentisic acid. Salicylic and gentisic and most of the phenolics are thought to occur in ester and glucoside forms (El Basyouni and Towers, 1963; Ribereau-Gayon, 1971). If not, ethanol would not extract them for analysis.

The results showed that both salicylic and gentisic have similar and divergent effects on wheat and barley plants grown in hydroponics. Both stimulatory and inhibitory effects as measured by increased and decreased plant heights and internode lengths were observed. These results were parallel to the findings of following workers: Varga and Koves (1959) in germination of legumes; Newmann (1959) in Helianthus hyphocotyl (excised) growth; Van Sumare et al. (1973) in germination and seedling growth of yeast, lettuce and barley; Gesto et al. (1967) in Avena coleoptile growth. Polyhydroxy phenolics, indeed, induced stimulatory growth effects at low concentration but inhibitory effects at higher concentrations (Newmann, 1959; and

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[1]Addition of benlate to control powdery mildew caused some chromosomal stickiness in the root tip cells. Sulfur dusting was employed from then on.

Nitsch and Nitsch, 1962). The findings may be explained by the affinity of salicylic acid and gentisic acid with the phytohormone balance in the plants (Kefeli and Kadyrov, 1971), particularly with the growth-promoting hormone, IAA (Cleland, 1963; Hare, 1964; Vieitz et al., 1967; and Basu, 1970). Zenk and Muller (1963) stated that the growth inhibitory and stimulatory effects of the phenolics were due to the inhibition and activation of the IAA oxidizing system. The changes in internode lengths suggested salicylic and gentisic acids might likewise have exerted their effects on plant growth by interfering with IAA metabolism.

The prolonged vegetative stage resulting in the delay of flowering might have been associated with the phenolic affinity for the phytohormone abscissic acid (ABA). The phenolic's inhibitory effect on nucleic-protein synthesis alters the metabolic system of the plant hormone (Kefeli and Kadyrov, 1971) necessary for flower initiation.

The observed continuous tillering probably was more of a function of the nutrient supply than of the imposed treatments (Aspinald, 1961 and 1963). A number of young tillers died and produced infertile spikes similar to the findings of Beaty et al. (1977) and Fletcher and Dale (1977).

ε-Amino Caproic Acid

Table 5 shows the effect of EACA on the height of barley and wheat plants grown in hydroponics. The hexaploid wheats were not affected by the different EACA concentrations. Uniform shoot growth was observed within each of the three stages of growth. The treated Cocorit plants were shorter than the controls; significant differences

Table 5. Effect of EACA on plant height (cm) of barley, tetraploid wheat and hexaploid wheat grown in hydroponics at three stages of growth

	Growth Stages	Concentration (ppm)			
		0 (Control)	1	10	100
H. vulgare cv. CM-67 (2n=14)	Fourth week	71.2a*	65.2b	65.0b	57.6c
	Sixth week	79.5a	90.0b	89.0b	82.0a
	Maturity	82.5a	76.5b	82.8a	77.2b
T. turgidum cv. Cocorit 71 (2n=4x=28)	Fourth week	50.4a	41.0b	45.0b	44.5b
	Sixth week	70.0a	59.0b	68.0a	63.5b
	Maturity	79.8a	79.2a	75.8a	75.5a
T. aestivum cv. Tobari (2n=6x=42)	Fourth week	56.1a	54.5a	56.2a	56.0a
	Sixth week	79.5a	77.0a	76.5a	71.5a
	Maturity	90.5a	74.0a	77.0a	77.0a

*Numbers followed by the same letter are not significantly different at 5% probability.

being observed during the fourth and sixth weeks of growth. Barley was most affected of the three test plants. At the fourth week, EACA inhibited the growth of barley seedlings because the treated plants were shorter than the controls. At the sixth week, however, the shoot growth was stimulated at 1 and 10 ppm; and at maturity, barley plants at 1 and 100 ppm were shorter than the controls.

The internode lengths of hexaploid wheat and barley showed no statistical differences among treatments at the three stages of growth. As mentioned above, the treated tetraploid wheats were shorter than the controls. At the fourth week, the controls had two elongated internodes while the treated Cocorits had only one and at the sixth week (see Table 6) all internodes of the control plants were longer than the internodes of the plants grown at 1, 10, and 100 ppms of EACA.

EACA did not affect the tillering capacity of hexaploid wheat and barley. The tetraploid wheat at 100 ppm of EACA produced fewer tillers than the control plants and plants grown at lower EACA concentrations. The tiller number was reduced by 1.5 tillers (significant at 5% probability level).

EACA significantly delayed the onset of flowering of barley and tetraploid wheat, but not of the hexaploid wheat. Flowering of barley was delayed by 7 days and 6 days at 1 and 100 ppms, respectively, but at 10 ppm was the same as the control, and that of the Cocorit by 2-3 days compared with the controls as shown in Table 7.

No chromosome abnormalities were observed in the studies of somatic and germ cells of the test materials (K. A. Mujeeb, personal communication).

Table 6. Effect of EACA on the internode length (cm) of barley, tetraploid wheat and hexaploid wheat at six weeks of growth in hydroponics

Concentration (ppm)	*H. vulgare* cv. Cm-67 (2n=14)					*T. turgidum* cv. Cocorit 71 (2n=4x=28)			*T. aestivum* cv. Tobari (2n=6x=42)		
	Internode*					Internode			Internode		
	1	2	3	4	5	1	2	3	1	2	3
0 (Control)	5.5a**	6.0a	9.0	10.5a	12.0a	8.0a	11.5a	12.0a	4.5a	9.0a	16.5a
1	3.0a	8.0a	14.0a	13.0a	11.0a	5.5b	7.0b	5.5b	5.0a	9.5a	17.0a
10	7.5a	7.5a	11.5a	12.0a	13.0a	7.0c	7.5b	4.0b	6.0a	9.0a	17.0a
100	5.0a	8.0a	8.5a	10.0a	11.0a	5.0b	8.5b	4.0b	5.0a	9.0a	16.0a

*Internodes are numbered from the base of the plant upward, number 1 is the basal internode.

** Numbers followed by the same letter are not significantly different at 5% probability.

Table 7. The effect of EACA on the onset of flowering (in days) of barley, tetraploid and hexaploid wheats grown in hydroponics

	Concentration (ppm)			
	0 (Control)	1	10	100
H. vulgare cv. CM-67 (2n=14)	29.5a*	36.0b	29.5a	35.0b
T. turgidum cv. Cocorit 71 (2n=4x=28)	47.0b	49.0b	49.0b	50.0c
T. aestivum cv. Tobari (2n=6x=42)	37.0a	36.5a	35.0a	35.0a

*Numbers followed by the same letter are not significantly different at 5% probability.

Germination tests on seeds from EACA-treated plants showed no chlorophyll mutants but germination rates decreased in all test plants at 100 ppm concentrations only. Germination rates results are shown in Table 8.

Traces of EACA were found in the leaf samples analyzed from all concentrations after the fourth week of EACA treatment. After six weeks, the analysis of leaf samples showed no traces of EACA but unknown peaks prior to lysine (between EACA and lysine positions) were observed. In relation to EACA, the other amino acids, lysine, histidine, and arginine, were variable with respect to the increasing/decreasing trends along the increasing concentration treatments as shown in Table 9.

This EACA experiment included a pot for 1000 ppm to observe if this EACA concentration was toxic. After a week of growth, chlorosis was noted; the same chlorotic symptom which had been observed in all seedlings at the lower concentrations of EACA. Addition of 2 ml of iron (6% $Fe_2SO_4 \cdot 7H_2O$-4% tartrate) everyday at the early growth period overcame chlorosis. The role of iron in chloroplast formation and chlorophyll synthesis in citrus and pineapple leaves was discussed by Walliham (1955), Sideres and Young (1956), and Jacobson and Oertli (1956). The latter authors, Jacobson and Oertli (1956), concluded that iron is involved in chloroplast formation through protein synthesis. The higher amount of iron requirement of EACA treated species probably indicates the involvement of EACA in protein synthesis (i.e., chloroplast formation and chlorophyll synthesis) which may be equated to the action of EACA on enzymes in the animal system. Thus, the stunted growth of barley and tetraploid

Table 8. The effect of EACA on the percentage germination rate of the first generation seeds of barley, tetraploid and hexaploid wheats grown in hydroponics

	Concentration (ppm)			
	0 (Control)	1	10	100
H. vulgare cv. CM-67 (2n=14)	98.90a	98.90a	98.90a	92.80b
T. turgidum cv. Cocorit (2n=4x=28)	98.90a	86.67b	78.08bc	71.08c
T. aestivum cv. Tobari (2n=6x=42)	96.10a	98.40a	90.30ab	88.30b

*Numbers followed by the same letter(s) are not significantly different at 5% probability.

Table 9. The effect of EACA on the basic amino acid contents (10^{-6} grams) of barley, tetraploid and hexaploid wheats grown in hydroponics

	Concentration ppm	Amino Acids			
		EACA	Lysine	Histidine	Arginine
H. vulgare cv. CM-67	0	0	232.737	127.058	128.395
	1	0	208.192	60.065	96.857
	10	0	204.508	72.687	91.101
	100	0	177.275	83.834	109.804
T. turgidum cv. Cocorit 71	0	0	309.983	81.211	158.626
	1	0	262.267	87.095	147.880
	10	0	221.209	54.094	92.094
	100	0	319.716	66.974	144.710
T. aestivum cv. Tobari	0	0	236.959	86.919	140.820
	1	0	256.079	82.209	126.572
	10	0	259.247	91.995	149.743
	100	0	232.737	135.564	211.674

wheat and the general inhibitory effect of EACA on total plant development might be attributed to alteration of protein synthesis (es) role of EACA.

It should also be considered that amino acids and their analogues inhibit seedling growth. Normal plant metabolites including amino acids were found to be inhibitory and toxic to the growth of Naturtium (Hofbuer and Minar, 1968); cress (Ausdus and Quastel, 1947); mung bean (Suda, 1960; and Smith and Fowden, 1966); and wheat and barley (Harris, 1956; Jalali and Suryanarayana, 1970; Green and Phillips, 1974; and Sing and Widholm, 1975). Growth inhibiting effects of amino acids were correlated with the inhibition of protein synthesis (Webster, 1955; Joy and Folkes, 1965; Dunham and Bryan, 1971; and Green and Phillips, 1974).

Hirono and Redei (1966) reported the acceleration of flowering of long-day plants with the application of the nucleic acid analogue, 8-azaadinine. However, flowering was inhibited by the addition of the nucleic acid analogue, 2 - thiouracil, to winter cereals (Suge and Yamada, 1968) and to rice (Inouye, 1965). Teltscherova et al. (1967) indicated that it was not clear whether the nucleic acid analogues specifically inhibited flowering or only generally suppressed growth and development in wheat plants. These reports of results with wheat and rice may be relevant to present results of inhibited growth and development of barley and tetraploid wheat treated with EACA and the resulting delay in flowering.

An EACA - lysine experiment was conceived upon noticing the apparent inhibitory effect of EACA on seedling growth of barley and wheat plants in hydroponics. The EACA - lysine experiment was done

in a supported hydroponic system. The prime objective was to determine if lysine alleviated the inhibitory effect of EACA. As shown in Table 10, the shortest barley and wheat plants were observed in the 1000-240 ppms of EACA - lysine added simultaneously. The growth of wheat was affected more than the growth of barley. The growth differences (i.e., against the control) were evident in wheat at 500 ppm and 1000 ppm of EACA alone or in combination with lysine. It should be noted, too, that the average response of the plants to EACA and lysine added singly was significantly different than when they were added together. The later addition of lysine to the 1000-240 ppm combination was less detrimental to the plants. The results were contrary to the reported lysine-reversal of growth inhibition in corn and pea seedlings of another of its analogues S-(2-aminoethyl)-L cysteine by Singh and Widholm (1975).

Later in the experiment, gibberellic acid (GA_3) was added to the EACA - lysine series. Gibberellic acid is known to promote cell elongation and enhance cell division. Saches et al. (1959) found GA_3 applied to the vegetative stage of plants, Hyoscyamus and Samolus, increased mitotic activity with increased stem length. Daufman (1965) reported the increased internodes of Avena (excised shoot) by promoting longitudinal growth. Both cell elongation and cell division promotion effects of GA_3 were reported by Bachelard (1969) in E. camaldulensis. Snir and Kessler (1975), in their study of GA_3 effect on some 46 plant species, concluded that an inverse relationship existed between the cellular DNA content and gibberellin sensitivity. The hexaploid wheats were less sensitive than the barleys to GA_3 addition. As shown in Figure 2, GA_3

Table 10. Effect of EACA and L-lysine on the plant height (cm) of barley and wheat grown in supported-hydroponics

	EACA-L-lysine concentration (ppm)						
	0/0 (Control)	100/0	500/0	1000/0	0/240	1000/240	1000/240*
H. vulgare cv. CM-67 (2n=14)	51.6a**	54.9a	55.4a	52.4a	53.0a	46.5b	51.2a
T. aestivum cv. Tobari (2n=6x=42)	47.9a	46 a	42.4a	39.7b	46.6b	35.4b	44.2a

*240 mg of L-lysine was added a week after the addition of EACA, thereafter added together.

**Numbers followed by the same letter are not significantly different at 5% probability level.

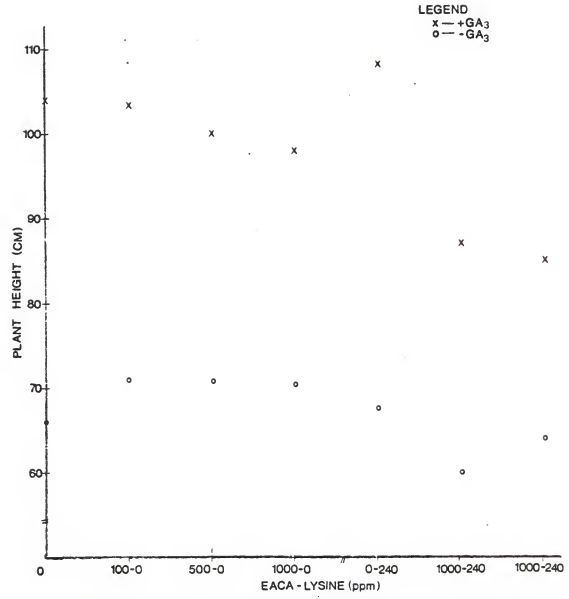

Figure 2. Effect of EACA-lysine with and without GA_3 addition on plant height of barley grown in supported hydroponic.

dramatically increased the height of barleys, however, those treated with 1000-240 ppm did not reach the height of the control plants. GA_3 did not reverse the initial inhibitory effect of EACA - lysine. Nitsan and Lang (1965) reported that decreased growth rate caused by the growth inhibitors was irrespective of exogenous GA_3. They suggested the occurrence of a non-competitive type of inhibition between GA_3 and the growth inhibitors.

Another EACA - lysine experiment was conducted in the straight (or unsupported) hydroponics which included equal molar concentrations of EACA and lysine. Only the wheats were used as test plants in this experiment. Plants grown at the different EACA - lysine levels were shorter than the controls at four and six weeks of growth as shown in Table 11. At the fourth week of treatment, the shortest plants were observed at the treatment with 1000/559 ppms of EACA - lysine added simultaneously. The shoot growth of hexaploid wheats was significantly different compared to the controls at the individual concentrations of EACA and lysine and at the combined levels. The growth differences were also observed in the Cocorit except at 100 ppm of EACA during both determinations. At six weeks, the hexaploid wheats showed significant differences in height expressions only at combined EACA - lysine concentrations, although those grown with EACA (or lysine) alone were shorter than the controls.

Addition of GA_3 overcame the effect of EACA - lysine in the case of Cocorits but not completely in the case of the Tobaris. The effect of EACA and lysine each added individually was overcome by GA_3 but the effect of EACA and lysine in combination was not, indicating that EACA - lysine in combination more severely inhibited the growth of

Table 11. Effect of EACA-L-lysine on the plant height (cm) of tetraploid and hexaploid wheats grown in hydroponics

		EACA-L-lysine concentration (ppm/ppm)							
		0/0 (Control)	100/0	500/0	1000/0	0/559	500/559	1000/559	1000/559*
T. turgidum cv. Cocorit 71	Four weeks	41a**	37.7a	28.8b	30.4b	31.0b	20.1c	13.0d	23.3c
(2n=4x=28)	Six weeks	58.7a	56.7a	33.5c	45.7b	49.3b	32c	18d	31c
T. aestivum cv. Tobari	Four weeks	48.5a	44.8b	34.3c	35.8c	40.2b	24.9d	20.0d	26.1d
(2n=6x=42)	Six weeks	67.0a	63.7a	60.0a	55.0a	56.7a	41.5b	38.76b	32.0b

*The 559 ppm of L-lysine was added a week after the addition of EACA, thereafter added together.

**Numbers followed by the same letters are not significantly different at 5% level.

hexaploid wheats as shown in Table 12.

The inhibitory effects of lysine and EACA on four-week-old tetraploid and hexaploid wheat seedlings might be associated with the decreased protein synthesis and alteration of the overall metabolic pattern of the plants as concluded by Dunham and Bryan (1971).

The beneficial effect of GA_3 on the tetraploid in all EACA treatments, alone or in combination with lysine, seems to agree with Shamarao and Kada (1974). The increased height of the hexaploid wheat with EACA and lysine added singly and that of the tetraploid wheat mentioned above follows the findings of Kaufman et al. (1965), Bachelard (1969), and Boeken and Van Oostveldt (1977). These workers reported GA_3 induced cell elongation and cell division.

Snir and Kessler (1975) also summarized a number of conflicting reports about GA_3 responses in plants due to the different ways of application, plant age, developmental stages, and number of treatments. They indicated that GA_3 had an inverse relationship with the cellular content of DNA and that dwarf mutants of corn, rice, and barley were highly sensitive to GA_3.

Chloramphenicol

Chloramphenicol (CAP) concentrations of 100 and 1000 ppms in hydroponics were toxic to barley and wheat seedlings. The first symptom was chlorosis of the youngest leaves. Within a week after initiation of the 1000 ppm treatment, the leaves of seedlings were completely devoid of pigmentation and dessicated, indicating cessation of chlorophyll formation or chlorophyll destruction or both. Plants

Table 12. Effect of EACA-L-lysine on plant height (cm) of tetraploid and hexaploid wheats in hydroponics, with and without GA_3 addition

		EACA-L-lysine concentration (ppm/ppm)							
		0/0 (Control)	100/0	500/0	1000/0	0/559	500/559	1000/559	1000/559*
T. turdigum cv. Cocorit 71 (2n=4x=28)	$-GA_3$	58.7a**	56.7a	33.5c	45.7b	49.3b	32c	18d	31c
	$+GA_3$	59.0a	61.0a	65.0a	55.0a	59.0a	55.0a	52.0a	52.0a
T. aestivum cv. Tobari (2n=6x=42)	$-GA_3$	67.0a	63.7a	60.0a	55.0a	56.7a	41.5b	38.7b	32.0b
	$+GA_3$	76.0a	78.0a	72.0a	66.0ab	71.0a	56.0b	57.0b	56.0b

*The 559 ppm of L-lysine was added a week after the addition of EACA, thereafter added together.

**Numbers followed by the same letters are not significantly different at 5% level.

treated with 100 ppm CAP survived longer than the 1000 ppm - treated plants, but the same chlorosis pattern and seedling deaths were apparent. Root tips were collected and investigated; there was no mitotic activity observed (K. A. Mujeeb, personal communication). Plants treated with 10 ppm CAP survived but discoloration of the youngest leaves was noted at an early stage of treatment. After four weeks, the leaves were observed to be normal but the seedlings were stunted.

Ten ppm of CAP significantly decreased the plant height of barley and that of the tetraploid wheats at maturity as shown in Table 13. Although the heights of the hexaploid wheat were not significantly decreased, visual observations indicated the Tobari plants had smaller culm diameter than the control. Tillering capacity of the test plants was not affected significantly as shown in Table 13. Onset of flowering, chromosome behavior, and germination rate of the harvested seeds were not affected by 10 ppm of CAP. No chlorophyll mutants were produced during the germination test.

The inhibitory effect of growth regulators was found to be directly proportional to the concentration (Allard, 1946). The survival time of the plants attested to this. Margulies (1962) equated the inhibitory growth effect of chloramphenicol on bacteria to the protein synthesis inhibition of CAP in higher plants. From the review of literature, the inhibitory effect on protein synthesis has been associated with the 70 s ribosome of the chloroplast, thus affecting chlorophyll formation and photosynthesis (Shlyk and Kostyuk, 1972, 1973). Simola (1973) further stated that:

Table 13. The effect of chloramphenicol on plant height (cm) at maturity, and tiller number of barley, tetraploid and hexaploid wheats grown in hydroponics

	Concentration mg/l	Plant height 0 (Control)	Plant height 10	Number of Tillers 0 (Control)	Number of Tillers 10
H. vulgare cv. CM-67 (2n = 14)		52.2a*	43.5b	4.5a	4.2a
T. turgidum cv. Cocorit (2n=4x=28)		63.8a	51.2b	4.0a	3.2a
T. aestivum cv. Tobari (2n=6x=42)		64.5a	58.0a	4.2a	2.2a

*Numbers followed by the same letter are not significantly different at 5% level.

> "The antibiotics act as virtual specific inhibitors of nucleic acid metabolism and protein synthesis, it follows that the development of cells of plants chiefly depends on the function of the cytoplasmic ribosomes and that the chloroplasts and mitochondria can be formed without synthesis of RNA in the nucleus or in presumed organell initials. The development of chloroplast and the synthesis of chlorophyll is dependent of the protein synthesis in 70 s ribosomes."

Acriflavine

Tests with acriflavine in hydroponics showed this dye to be highly toxic to the barley and wheat seedlings. Two days after the different concentration treatments, the seedlings absorbed the greenish-orange dye. The hue was darker at 100 ppm and 1000 ppm of acriflavine compared to the lower concentration of 10 ppm. Death of seedlings occurred early, even at 10 ppm, the lowest concentration tested. Acriflavine has been reported to inhibit growth of rice and corn coleoptiles due to the formation of a DNA-dye complex (Kou, 1969). The DNA-dye complex might have been the cause of the total and complete inhibition observed in the test plants.

Injection and Spray Experiments

Four of the five immunosuppressants tested in hydroponics were used in these experiments. They were EACA, gentisic acid, chloramphenicol and salicylic acid. Injection and spray experiments were done in the greenhouse with test plants at booting stage. The different delivery methods - injection, aqueous spray with and without a carrier - were compared with respect to spike development (weight of spikes) and number of seeds produced. Chromosome behaviour and germination test were investigated as in the hydroponic experiments.

Leaf burns were observed in barley plants--Promesa and Porvenir--sprayed with carrier and carrier control. The injury was attributed to the common factor of the treatments - the presence of 0.5% Tergitol S-15 as the surfactant carrier agent.

Leaf burning was also observed in plants treated with salicylic acid. In all delivery methods, leaves of wheat and barley plants became necrotic and senesced and the plants ultimately died. The toxic effect of salicylic acid was also observed in hydroponics.

Chloramphenicol delivered through injection caused chloroses of leaves of wheat and barley plants. Porvenir plants died after injection and aqueous spray application of chloramphenicol. Degradation and inhibition of syntheses of chloropyll by chloramphenicol is described in the hydroponic experiments. Poor spike development and no or lower seed production effect of CAP are shown in Tables 14 and 15.

Gentisic acid and EACA caused better (though not significantly) spike development and increased seed set when applied as aqueous

Table 14. Mean weight (g) of barley and wheat spikes using the different methods of chloramphenicol introduction to plants

	Control	Injection	Aqueous Spray	Spray with Carrier	Carrier Control
T. aestivum cv. Tobari	1.30	0.26	1.28	0.56	1.11
T. turgidum cv. Cocorit 71	1.33	0.39	1.33	0.91	1.27
H. vulgare					
cv. Promesa	0.96	0.68	1.23	0.51	0.81
cv. Porvenir	1.11	-	-	0.84	0.73

Table 15. Mean seed number per spike of barley and wheat plants using the different methods of chloramphenicol introduction to the plants

	Control	Injection	Aqueous Spray	Spray with Carrier	Carrier Control
T. arestivum cv. Tobari	22	-	23	19	27
T. turgidum cv. Cocorit 71	23	no seed	24	19	28
H. vulgare					
cv. Promesa	22	11	30	15	25
cv. Porvenir	27	-	-	18	15

sprays than when injected or applied as spray with carrier (Tables 16-19). The only treatment that resulted in death of plants was injection of Porvenir with gentisic acid.

No chromosome abnormalities were detected in plants subjected to the different methods of introduction nor from the chemicals themselves (K. A. Mujeeb, personal communication). Germination of seeds from treated and control plants were not statistically different. Seedlings were vigorous and without chlorosis.

Aqueous sprays of immunosuppressants have advantages over the most accepted method of chemical introduction to the plants-injection as a practical field technique. Not only are aqueous sprays time-saving and less tedious but they also eliminate the mechanical injuries to the plants caused by injection. The use of a carrier in spraying was also harmful because of the leaf burns and a seemingly synergistic effect with the chemicals on growth inhibition.

Table 16. Mean weight (g) of spike of wheat and barley plants using different methods of EACA introduction to the plants

	Control	Injection	Aqueous Spray	Spray with Carrier	Carrier Control
T. aestivum cv. Tobari	1.53	1.22	1.26	1.39	1.42
T. turgidum cv. Cocorit 71	1.84	1.56	1.71	1.85	1.53
H. vulgare					
cv. Promesa	0.89	1.01	0.78	0.76	0.71
cv. Porvenir	1.58	1.67	1.29	1.44	1.52

Table 17. Mean number of seeds per spike of barley and wheat using the different methods of introduction of EACA

	Control	Injection	Aqueous Spray	Spray with Carrier	Carrier Control
T. aestivum cv. Tobari	28	28	23	26	27
T. turgidum cv. Cocorit	27	23	28	27	27
H. vulgare cv. Promesa	20	20	17	17	15
H. vulgare cv. Porvenir	37	29	29	30	35

Table 18. Mean weight (g) of barley and wheat spikes using the different methods of gentisic acid introduction to the plants

	Control	Injection	Aqueous Spray	Spray with Carrier	Carrier Control	LSD 0.05
T. aestivum cv. Tobari	1.21	0.95	0.95	0.95	1.14	
T. turgidum cv. Cocorit 71	1.24	1.09	1.19	0.80	0.79	
H. vulgare						
cv. Promesa	0.89	0.51	0.91	0.72	0.68	
cv. Porvenir	1.45	-	1.01	0.65	1.09	0.44

Table 19. Mean seed number of wheat and barley spikes using the different methods of introduction of gentisic acid

	Control	Injection	Aqueous Spray	Spray Carrier	Carrier Control
T. aestivum cv. Tobari	24	15	21	17	24
T. turgidum cv. Cocorit	25	19	20	15	14
H. vulgare					
cv. Promesa	22	13	23	18	21
cv. Porvenir	35	-	21b	15b	29c

SUMMARY

Successful use of immunosuppressants in overcoming the crossability barrier(s) in cereal wide crosses is highly dependent on an efficient delivery system to the plants. It is also necessary to understand how the fine immunosuppressants affect the growth of wheat and barley.

Salicylic acid, gentisic acid, EACA and chloramphenicol at concentrations tolerated by wheat and barley did not alter the chromosome behaviour of either somatic or germ cells (K. A. Mujeeb, personal communication).

In hydroponics, high concentrations (1000 and 100 ppms) of salicylic acid and chloramphenicol and acriflavine at low and high concentrations (10, 100, 1000 ppm) were toxic to wheat and barley seedlings. One hundred ppm of EACA and gentisic acid inhibited growth.

EACA, gentisic acid and salicylic acid showed stimulatory as well as inhibitory effects on growth in terms of plant height and internode lengths. In most cases, flowering was delayed but tillering was not affected except by EACA at 100 ppm.

EACA alone or in combination with lysine inhibited wheat growth. The inhibitory effects of high concentrations of EACA-lysine were not overcome by the addition of GA_3. Inhibitory and toxic effects of the immunosuppressants were attributed to alteration of protein synthesis and metabolism of plant reactants by the chemicals.

Foliar sprays of aqueous solutions of the chemicals were advantageous as compared with injection methods and/or the use of a sur-

factant carrier. They were less time-consuming, less tedious and less injurious to the plants.

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EFFECTS OF IMMUNOSUPPRESSANTS ON PLANT GROWTH AND DEVELOPMENT

by

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The transfer of a known high lysine gene of barley to wheat would enhance the nutritional status of wheat products. The success of the intergeneric cross between rye and wheat (Triticale) was not achieved in the cross between barley and wheat, indicating the presence of a crossability barrier. The incompatibility mechanism(s) has been suggested to be similar to the immunochemical system in animals and has been termed stereospecific inhibition reaction (SIR) in plants. The potential use of five immunosuppressant chemicals (salicylic acid and its phenolic compound, gentisic acid; ε-amino caproic acid (EACA); chloramphenicol; and acriflavine) to overcome the crossability barrier in cereal wide crosses has been advanced through the SIR theory.

The effects of the above chemicals on barley and wheats were studied. Various application methods--hydroponics, injection and aqueous spray with and without a surfactant (Tergitol S-15), were tested to determine their effect on chromosome structure, gross plant morphology and on uptake of the chemicals by the plants.

Salicylic acid and chloramphenicol caused toxic effects at high concentrations in hydroponics and injection methods. Acriflavine was tested only in hydroponics and found to be highly toxic to wheat and barley seedlings in all concentrations used. Gentisic and EACA were not toxic, but induced growth inhibitory effects in hydroponics.

Somatic cells and germ cells showed no chromosomal abnormalities that could be attributed to the chemicals. Germination of seeds from treated plants was not affected, except by EACA, which decreased germination at high concentration in hydroponics. No chlorophyll

mutants were produced during the germination test.

The inhibitory effects of the chemicals on plant growth and development in hydroponics were possibly due to biochemical changes induced by them. They suggested metabolic patterns of the plants were altered and inhibited, particularly nucleic acid and protein synthesis. The hexaploid wheats were the least affected among the test plants.

EACA in combination with lysine severely inhibited the growth of wheat and barley. The addition of gibberellic acid to EACA-lysine combination treatments did not overcome the growth inhibition.

These experiments serve as baseline studies in an understanding of immunosuppressants' actions in plants.