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Running title: **Effects of Disinfectants in Water on Mir- and Earth-grown Wheat**

Mir Post-Flight Studies on The Effects of Disinfectants in Water on Plant Growth of Wheat

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ABSTRACT

Iodine and silver fluoride are used to purify water onboard U. S. Shuttles and the Russian Space Station, Mir, respectively. In 1995, iodine-treated water, which ranged from 1.0-4.0 mg · kg^{-1} with a mean of 2.9 mg · kg^{-1}, was applied to Super Dwarf wheat (*Triticum aestivum* L.) plants when Mir water (grey or tech grade) became scarce. The potential phytotoxicity of iodine on Super Dwarf wheat is an unknown. Since use of iodine-treated water was not part of the experiment, we sought to determine whether it accounted for the subsequent poor wheat seedling growth and floral development onboard the Mir. Super Dwarf wheat seeds were imbibed in iodine or silver fluoride concentrations of 0.0, 1.0, 2.0, 4.0, 8.0 or 16.0 mg · kg^{-1} for 96 h at 4 °C. Five seeds were then planted per 13.3 cm x 13.3 cm pots containing a granular clinoptilolite (Cp) zeolite (1-2 mm dia.) and placed in Percival™ growth chambers programmed for 20/15 °C and 18/6 h d/n regime. Plants were irrigated with distilled water, and Iodine- or silver fluoride-treated distilled water. In separate experiments, seeds were treated as above and germination and early seedling growth were determined by examining seedling responses to disinfectants in rolled paper towels. Silver fluoride had very little effect on wheat seed germination. By contrast, iodine reduced germination at all treatment levels. Seedlings exposed to 1.0, 2.0, and 4.0 mg · kg^{-1} of iodine or silver fluoride levels exhibited a slight stimulation in shoot and root growth. Both disinfectants at 8 and 16 mg · kg^{-1} showed significantly ($p \leq 0.01$) reduced seedling shoot and root lengths and fresh biomasses compared to the control and lower disinfectant levels. The number of spikelets per spike, florets per spikelet, seeds per spike and seed weight were also significantly reduced at the 8 and 16 mg · kg^{-1} compared to the control and lower levels of disinfectant. Based on these ground-based post-flight analyses, the levels of iodine- and/or silver fluoride-treated water used on Mir-grown plants onboard the Mir did not cause the poor growth and development of the wheat plants.

Key words: *Super Dwarf Wheat germination, iodine, silver fluoride, purified water, seedling growth, Mir, Space Biology, Microgravity*

INTRODUCTION

Water used onboard U. S. Shuttles is processed though a microbial check valve (MCV) that regulates the influx of iodine used as a disinfectant to purify the drinking water, whereas the Russians used silver on the Space Station, Mir. When shuttles disengaged from the Mir, all available water and consumable supplies were left onboard for the Mir crew. Six weeks into the wheat experiment conducted on Mir in 1995, the crew members who tended the Space Lab Module-1 Greenhouse-2 study irrigated the wheat plants with iodine-treated water (Salisbury, 1997). The iodine concentration of the water used to irrigate the wheat plants ranged from 1.0-4.0 mg · kg^{-1} with a mean concentration of approximately 2.9 mg · kg^{-1}.

Although iodine is an essential nutrient element for humans and animals, relatively little is known of its importance to plants or its availability for uptake by plants (Jopke et al., 1996; Underwood, 1966). By contrast, many kinds of marine algae contain iodine as an essential element, which may reach concentrations of 10 mg · kg^{-1} in their dry matter intake, a concentration 30,000 times higher than seawater. The geological origin of soil may influence the iodine content in plants, which depends on its concentration in soils, depositions, air and fertilizer (Anke et al. 1993; Macnicol and Beckett, 1985). Also, it has been demonstrated that a plant variety may show different iodine contents depending on ecological conditions during its growth phases with a range varying from 0.07-1.2 mg · kg^{-1} of dry matter, the iodine being preferably stored in vegetative tissue, i.e., leaves and stems (Jopke et al., 1996). For iodine fertilization, the capability of the soil to absorb iodine is decisive. In clay and humus soils, the iodine is fixed, therefore, the content in plants tend to be relatively low, but in sandy soils, toxic levels readily accumulate in some plants, e.g., leaves of tomato readily show chlorotic spots and roll up and die (Bors and Martens, 1992; Jopke et al., 1996; Yuita et al., 1991).

Working with seeds of barley (*Hordeum vulgare* L.), tomato (*Lycopersicum esculentum* L.) and pea (*Pisum sativum* L.), Umaly and Poel (1970) noted that iodine supplied as potassium iodide enhanced the height and fresh and dry weights of barley and tomato at iodine concentrations of 0.5 and 1.0 mg · kg^{-1}. At these levels, they also noted that barley plants exhibited an increase in number of tillers. Both species exhibited well-developed root systems and the leaves were more numerous, larger and of a richer green color. When treated with 5.0 mg · kg^{-1} of iodine, these two species were indistinguishable from their controls, whereas 10.0 mg · kg^{-1} was inhibitory to growth and induced symptoms of toxicity such as general chlorosis, yellow interveinal patches and brown necrotic spots. With pea, all iodine treatments reduced growth, and the magnitude of effect and severity of toxicity symptoms increased with increased concentration.

Sheppard and Evenden (1992) reported that iodine is effectively retained in soils and may attain levels toxic to plants. In a series of field plot experiments, they observed control fresh-weight plot yields of 690 g m^{-1} for red beet (*Beta vulgaris* L., `Detroit Dark Red') root, 1,000 g per cabbage (*Brassica oleracea* var. *capitata* Golden Cross) head, 680 g $m^{-1}$ of corn (*Zea mays* L. `Spartan') ears, and 1490 g m^{-1} of corn stover. They noted that high iodine treatments (125 mg · kg^{-1}) lowered beet root and corn stover yields, to 300 and 110 g m^{-1}, respectively, whereas the corn ears were not significantly different from the controls. By contrast, beet root yields were significantly lowered to 370 g m^{-1} by low iodine treatment (50 mg · kg^{-1}).

Fluorine, the lightest, and iodine, the heaviest, of the halide elements are both quite toxic to plants at relatively low levels and are considered industrial pollutants (Robinson, 1978). However, Elrashidi and Lindsay, (1987) reported that fluorine application to soils can solubilize nutrients and increase plant yields. Plants readily and commonly absorb fluorine from the atmosphere as well as the soil, in which both sources may accumulate to levels toxic to plants (Sheppard and Evenden, 1992). These scientists further noted that the plant yields were not significantly different from the controls when plants were exposed to a mixture of iodine, fluorine, chlorine, and bromine. These responses suggest competitive interactions among the mixture of halides, i.e., the added halides decreased the toxicity of iodine. A number of scientists have reported competition during plant uptake between halides and nitrate (van Eysinga and Spaans, 1985), orthophosphate (Kundu et al., 1987) and sulphate (Schnug and Schnier, 1986).

Bubenheim and Patterson (NASA/Ames Research Center, personal communication, 1997), working with iodine-treated water ranging from 0.5 to 8.0 mg $\cdot$ kg^{-1} on lettuce,(*Lactuca sativa* L.) seedlings, concluded from their results that the iodine level used onboard Mir would probably have no potential deleterious effects on growth of wheat during the Svet (means "light" in Russian) greenhouse experiment. However, results from a series of ground-based pilot experiments showed that Super Dwarf wheat seedlings may be sensitive to iodine-treated water in the upper range used onboard the Mir Station. Moreover, use of the iodine-treated water was not part of the experiment and the potential phytotoxicity is an unknown.

Our objective in post-flight analysis of environmental constraints to which wheat plants were exposed onboard Mir was to determine whether iodine- or silver fluoride-treated water would inhibit seed germination and seedling growth parameters associated with discoloration of wheat leaves and failure of floral development (complete absence of spike formation) observed in Super Dwarf wheat growing onboard the Mir in 1995.

MATERIALS AND METHODS

Wheat (*Triticum aestivum* L.) cv Super Dwarf seeds were imbibed in varying concentrations (0.0, 1.0, 2.0, 4.0, 8.0 or 16.0 mg $\cdot$ kg^{-1}) of iodine as potassium iodide or silver fluoride at 4 °C for 96 h in parafilm sealed Petri dishes to alleviate post-harvest dormancy and to ensure germination. Five seeds were then planted per plastic pot (13.3 cm x 13.3 cm) in clinoptilolite (Cp), a natural zeolite 1-2 mm dia (referred to as Balkanine by our Bulgarian and Russian Team members). The pots were placed in trays containing distilled water and iodine- or silver fluoride-treated distilled water, and the trays placed in Percival™ growth chambers (Model E-54-U, Boone, IA 50036) programed for 20/15 °C temperature, a photosynthetic photon flux (PPF) of 400 μmol $\cdot$ m^{-2} $\cdot$ s^{-1} and 18/6 h d/n photoperiod regime was sufficient for the plants to carry out photosynthesis and acted as a beacon for plant shoots onboard space vehicles. Plants received irrigation treatments through holes in the bottom of the pots via capillary movement. The number of spikelets per spike, florets per spikelet, seeds per spike and seed wt (g) were monitored.

The Balkanine (zeolite) in which the plants were grown is unique in having cation-exchange, adsorption and hydration-dehydration properties (Ming and Mumpton, 1989; Boettinger and Graham, 1995; Perrin et al., 1998). Such traits allow the Balkanine, when nutrient-charged, to function as a slow release fertilizer and as a moisture-control agent, which has great potential as a vector for root growth in the microgravity of space.

Additionally, 50 seeds per treatment per experiment were incubated at 4 °C for 96 h as described above and placed in rolled paper towels, germinated and grown for 8 d at 25 °C to ascertain the effects of disinfectants on seed germination and shoot and root morphology and growth. Seedling growth parameters, i.e., shoot and root length and fresh biomass, were recorded. These experiments were repeated four times and laid out in a completely randomized block design with four replications. Collected data were subjected to standard analysis of variance according to the protocol of Federer (1955).

RESULTS AND DISCUSSION

The analytical data of iodine and silver fluoride on Super Dwarf wheat are shown in Tables 1 and 2. Except for the 16 $mg \cdot kg^{-1}$ treatment, silver fluoride only moderately reduced wheat seed germination (Table 1). By contrast, iodine significantly ($p \leq 0.01$) reduced seed germination at all treatment levels compared to the control plants. There were no significant differences in the root lengths of seedlings exposed to silver fluoride, whereas shoot lengths were significantly reduced at the 16 $mg \cdot kg^{-1}$. Shoot and root lengths exposed to 1.0 and 2.0 $mg \cdot kg^{-1}$ of iodine exhibited significant ($p \leq 0.01$) stimulation. However, both disinfectants showed significantly reduced seedling shoot and root lengths and fresh biomass at 8 and 16 $mg \cdot kg^{-1}$ compared to the control and lower disinfectant levels.

After 45 d of growth in the disinfectants, only the 16 $mg \cdot kg^{-1}$ of iodine treatment showed substantial reduction in plant growth (Fig. 1), and as the plants approached maturity, symptoms of toxicity such as general chlorosis and yellow interveinal patches were noted. Seedlings exposed to 16 $mg \cdot kg^{-1}$ of silver fluoride showed only a slight reduction in growth (Fig. 2). Silver fluoride required a treatment of 64 $mg \cdot kg^{-1}$ to the wheat seedlings to induce similar responses of the 16 $mg \cdot kg^{-1}$ iodine treatment. Wheat plants treated with lower levels of iodine were indistinguishable from the controls. These observations on Super Dwarf wheat were similar to those reported earlier by Umaly and Poel (1970) for barley plants that received 0.5, 1.0 and 5.0 $mg \cdot kg^{-1}$ of iodine. Our plant responses to iodine treatments of 8 and 16 $mg \cdot kg^{-1}$ were similar to those of Umaly and Poel's (1970) 10.0 mg · kg^{-1} treatment level. They noted brown necrotic spots on barley leaves to which they attributed to manganese toxicity. By contrast, our data differed from theirs in that we saw no differences in the number of tillers produced on wheat, whereas they reported six to nine tillers on iodine-treated barley compared to four in the controls. Umaly and Poel (1970) also noted that iodine-treated barley plants had well-developed root systems, whereas in wheat with one exception, we observed significant reductions in root biomass at the higher iodine treatment levels (Table 1).

At maturity and harvest, the number of spikelets per spike, florets per spikelet, seeds per spike and seed weight per spike significantly decreased ($p \leq 0.01$) as the level of disinfectants increased (Table 2). Although the 16.0 mg · kg^{-1} iodine-treated plants were struggling to stay alive after 45 d (Fig. 1), they succumbed at maturity (Table 2). Both disinfectants significantly decreased ($p \leq 0.01$) the seed yield components at the 8.0 mg · kg^{-1} level and higher.

Studies relative to higher plants, e.g., perennial ryegrass (*Lolium perenne* L.), have included the effects of iodine in various formulations (iodide, elemental iodine and iodate) added to soils in the field and in soil and nutrient solution cultures (Whitehead, 1975). In some cases, herbage yields were not affected by the addition of any of the three forms of iodine to the soil. By contrast, the addition of organic matter to the soil depressed growth, whereas when lime was applied to the soil, stimulatory effects on yields of herbage were observed. Iodoacetate also was stimulatory to tomato plants at low concentrations, but the roots were much less tolerant of this formulation, with 1 mg · kg^{-1} of iodine proving toxic. Also, iodine, supplied as potassium iodide or as methylene iodide, enhanced growth in concentrations up to 1 mg · kg^{-1} with an optimum at about 0.5 mg · kg^{-1}. In total absence of iodine, however, growth of tomato roots in tissue culture decreased during the transfer to successive sub-cultures, suggesting that iodine may be a required micro-nutrient.

These stimulatory effects and the unexpected high enrichment of iodine in cress (*Lepidium sativum* L.) observed by Jopke et al. (1996) may lead to the recommendation to produce cress and perhaps other crops in such a way that iodine can be a prophylactic for the consumer against iodine deficiency. Although current knowledge indicates iodine is an non-essential element for higher plants, plants can and do assimilate iodine. Thus, in long term space craft occupation, plant-supplied iodine may be crucial to the health of astronauts (Salisbury, 1999). Finally, from these ground-based studies, we conclude that the 1.0 to 4.0 mg · kg^{-1} level of iodine exposures that occurred in the Svet greenhouse onboard Mir most probably had no deleterious effects on wheat growth parameters or floral development.

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FIGURE LEGEND

FIGURE 1. Super Dwarf Wheat Seedlings' Responses after 45 d Exposure to (A = 0, B = 1.0, C = 2.0, D = 4.0, E = 8.0 and F = 16.0 mg L^{-1}) Iodine Applied as Potassium Iodide.

FIGURE 2. Super Dwarf Wheat Seedlings' Responses after 45 d Exposure to (A = 0, B = 1.0, C = 2.0, D = 4.0, E = 8.0 and F = 16.0 mg L^{-1}) Silver Fluoride.

Table 1. Effects of Iodine- and Silver Fluoride-treated Water on Super Dwarf Wheat Seed Germination, Shoot and Root Length and Fresh Biomass (Paper Towels, 8 d).

Level (mg/kg)	Seed Germ.[@]	Length (cm)		Fresh Biomass (g)	
		Shoot	Root	Shoot	Root
			AgF		
0.0	85 ab	11.4 a	9.5 d	3.1 a	3.7 a
1.0	85 ab	11.4 a	20.0 a	2.9 a	2.7 c
2.0	80 ab	11.2 ab	19.6 a	2.5 ab	4.3 a
4.0	90 a	10.7 b	15.1 b	2.2 b	3.4 a
8.0	83 b	10.8 b	14.7 bc	2.5 ab	3.1 b
16.0	78 b	9.7 c	14.2 c	2.3 b	3.0 b
			Iodine		
0.0	83 a	15.3 c	18.6 b	5.1 b	2.7 a
1.0	73 b	16.5 b	19.2 ab	4.7 b	2.3 b
2.0	59 d	17.8 a	20.9 a	7.3 a	3.2 a
4.0	64 c	14.8 c	17.5 c	5.0 b	1.9 c
8.0	59 d	10.0 d	10.9 d	1.0 c	1.4 c
16.0	43 e	8.7 e	11.4 d	1.4 c	0.8 d

[@]Different Letters Following Mean Numbers (n = 50 Seeds x 4 Replications x Four Separate Experiments) Within Treatments Indicate they are Significantly Different $p \leq 0.01$.

Table 2. Effects of Iodine and Silver Fluoride-treated Water on the Number of Spikelets per Spike, Florets per Spikelet, Seeds per Spike and Seed Weight per Spike of Super Dwarf Wheat

Level (mg/kg)	Spikelets/ Spike	Florets/ Spikelet	Seeds/ Spike	Seed Wt (g)/ Spike
		AgF		
0.0	20.3 b	3.0 a	14.6 a	2.6 b
1.0	20.8 b	3.1 a	10.3 b	2.5 b
2.0	22.6 a	2.5 b	14.5 a	2.4 b
4.0	21.3 ab	3.1 a	15.3 a	3.2 a
8.0	9.7 c	2.5 b	5.8 c	1.2 c
16.0	9.5 c	2.4 b	5.4 c	1.5 c
		Iodine		
0.0	22.0 a	3.1 ab	17.5 a	3.1 a
1.0	21.0 a	4.0 a	15.2 b	2.3 b
2.0	19.1 b	3.0 bc	13.8 c	2.5 ab
4.0	18.3 b	3.5 ab	13.8 c	1.6 c
8.0	15.2 c	2.8 c	7.0 d	0.8 d
16.0	0.0 d	0.0 d	0.0 e	0.0 e

[@]Different Letters Following Mean Numbers (n = 5 Seeds per Pot x 4 Replications x Four Separate Experiments) Within Treatments Indicate they are Significantly Different $p \leq 0.01$.

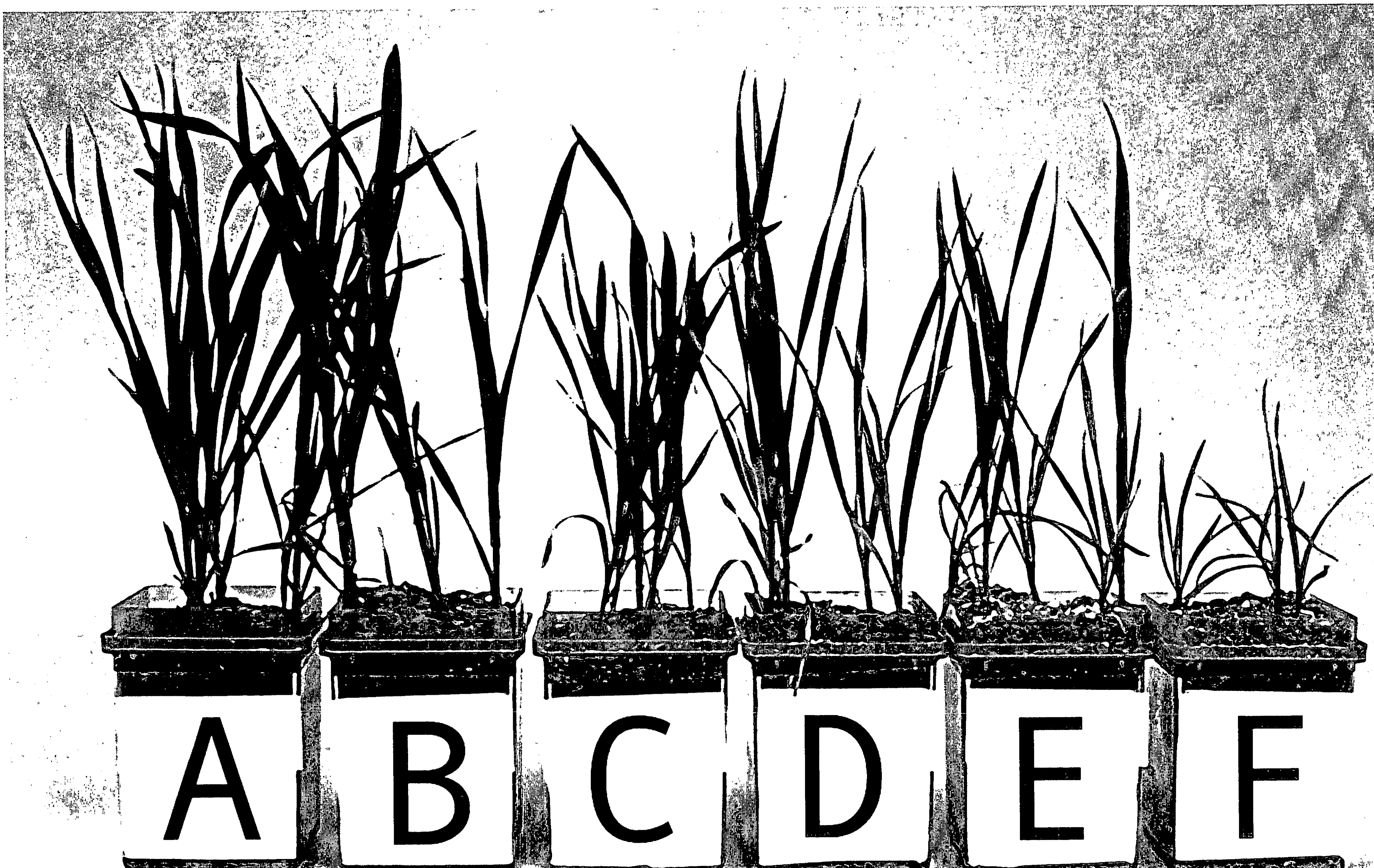

Fig. 1

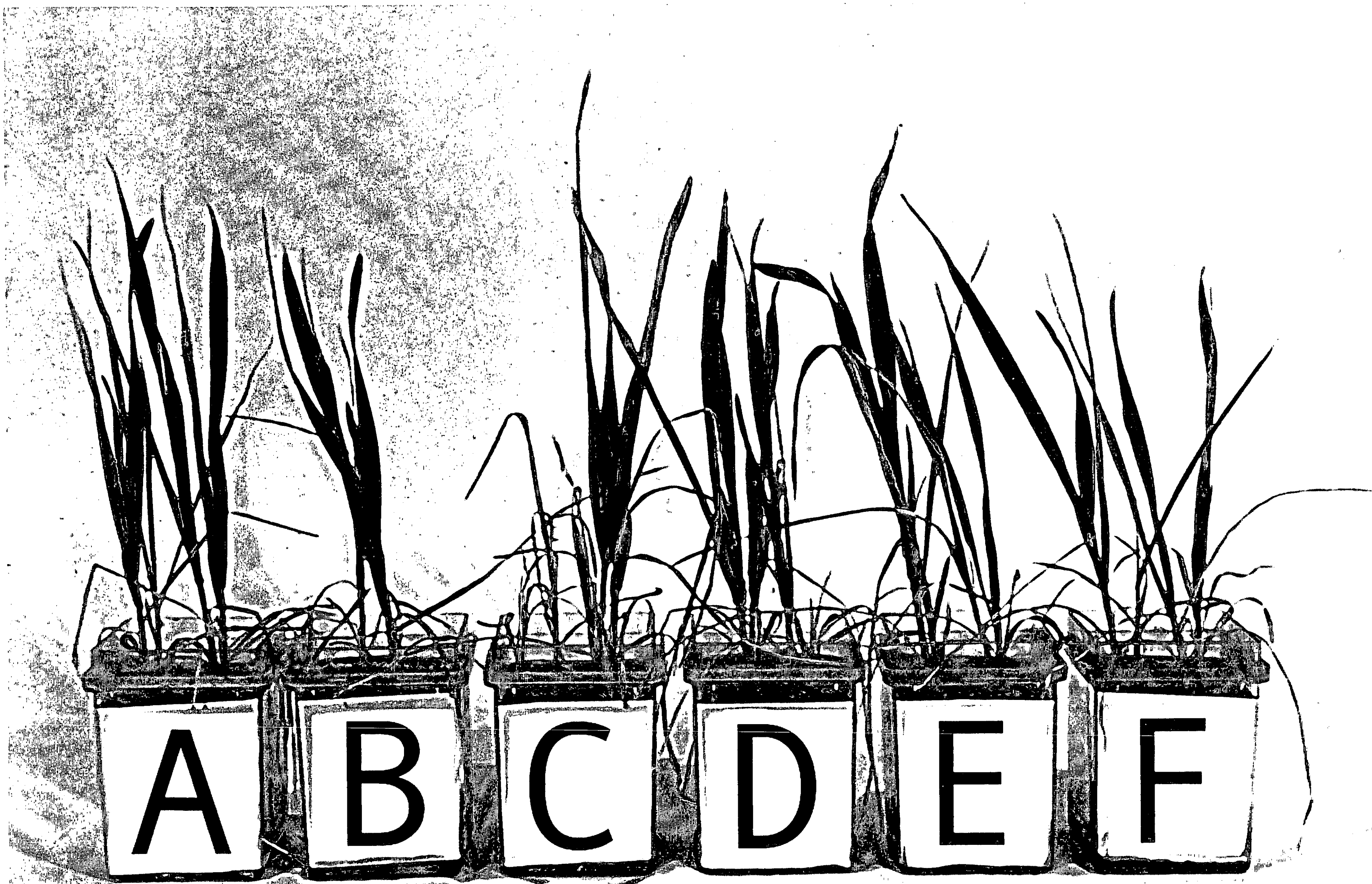

Fig. 2