Differential Response of Two Soybean Cultivars to Paraquat

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The soybean cultivars "Kwangkyo" and "Hood" are differentially sensitive to the bipyridylium herbicide paraquat (1,1′-dimethyl-4,4′-bipyridinium ion). This was confirmed by visible injury observations, measurements of desiccation levels and chlorophyll content, and tracings of chlorophyll fluorescence induction of fully expanded first trifoliate leaves of these two cultivars after exposure to a wide range of paraquat concentrations. The margin of this intraspecific differential tolerance to paraquat was narrow and the ratio of the paraquat concentrations causing 50% injury to the tolerant Kwangkyo and to the susceptible Hood soybean (approximate tolerance factor) was found to be 10. Paraquat at 1 μM or higher inhibited rapidly the CO_2 fixation capacity of leaf mesophyll cells, isolated enzymatically from both cultivars. Thus, the tolerance of Kwangkyo soybean to paraquat does not appear to result from any differences at the site of paraquat action in chloroplast membranes. At early time periods (30 min to 2 h) after treatment with 100 μM of paraquat, chlorophyll fluorescence induction was completely suppressed in first trifoliate leaves of Hood, but not in those of Kwangkyo soybean. At longer time periods (≧ 3 h), paraquat suppressed chlorophyll fluorescence induction similarly in leaves of both soybean cultivars. These results suggest that reduced mobility or a delayed release of paraquat in the mesophyll cells of Kwangkyo may be involved in the observed tolerance of this soybean cultivar to this herbicide.

Introduction

The herbicide paraquat is a nonselective contact herbicide causing a rapid desiccation of green tissues due to membrane damage [1, 2]. Paraquat competes for electrons with the primary electron acceptor of photosystem I and is reduced by such electrons forming a paraquat radical. The paraquat radical then transfers these electrons to molecular oxygen producing several oxygen species such as superoxide ion, hydrogen peroxide, and hydroxyl radicals which are very reactive and toxic [1, 2].

In recent years, resistance and/or tolerance to several herbicides has been documented in many weed species [1, 3]. In 1975, Faulkner [4] was the first to report on the selection of a line of perennial ryegrass (*Lolium perenne* L.), which was tolerant to paraquat. Since then, a growing number of paraquat-resistant biotypes from several weed species have been identified around the world [3–11].

To explain the development of tolerance and/or resistance of weed biotypes to the herbicide paraquat, two major mechanisms have been proposed [1, 2]. The first mechanism has correlated plant tolerance and/or resistance to paraquat with elevated levels and/or activities of the components of the chloroplast antioxidant system [1, 2]. Such components include the scavenging enzymes superoxide dismutase (SOD), ascorbate peroxidase, and glutathione reductase (GR) and antioxidants such as ascorbate, glutathione and α-tocopherol. According to the second theory, resistance to paraquat arises from limited movement or sequestration of the herbicide in tolerant and/or resistant weed biotypes [1, 2].

In field screening studies using 63 cultivars of soybean, Kim *et al.* [12] selected Kwangkyo as a paraquat tolerant cultivar. Hood and other soybean cultivars were described as susceptible.

The major objectives of the present research were to: a) compare the responses of Kwangkyo and Hood soybean to paraquat utilizing several assays, and b) characterize the margin of the differential response of these two soybean cultivars following treatments with the herbicide paraquat.

Materials and Methods

Chemicals

Formulated paraquat (GRAMOXONE®) and the surfactant X-77 were obtained from Chevron

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chemical company, Richmond, California. Analytical grade paraquat and other reagents were obtained from Sigma Chemical Company, St. Louis, Missouri. Macerase was obtained from Calbiochem, LaJolla, California, and $NaH^{14}CO_3$ was obtained from ICN Radiochemicals, Irvine, California.

Paraquat effects on Kwangkyo and Hood seedlings

Seeds of Kwangkyo and Hood soybean [*Glycine max* (L.) Merr.] were planted in 200 ml styrofoam cups filled with a mixture of peat moss, vermiculite and weblite (1:2:2, v/v/v) and grown in a greenhouse with 25 ± 5 °C, 16 h photoperiod and a photosynthetic photon flux density (PPFD) of 500 μE m^{-2} s^{-1} provided by low pressure sodium lamps. Upon reaching the stage of the first fully expanded trifoliate, soybean seedlings were sprayed with 0, 1, 10, 100, 500, and 1000 μM paraquat. The herbicide solutions contained 0.24% of the nonionic surfactant X-77 and were sprayed with a hand atomizer until run-off. Sprayed seedlings were placed in a growth chamber with 25 ± 5 °C temperature and continuous light of 600 μE m^{-2} s^{-1} PPFD. At 24 h after treatment with paraquat, visible injury on soybean seedlings of both cultivars was evaluated using the scale of Gullner *et al.* [13], whereas percent desiccation was determined according to the method of Finckh and Kunert [14]. There were three replications of each treatment, and the experiment was repeated twice.

Paraquat effects on chlorophyll content of excised trifoliates of Kwangkyo and Hood soybean

First fully grown trifoliates were excised under water from seedlings of both soybean cultivars, grown as described in the previous section, and dipped into 20 ml vials containing paraquat at 0, 1, 5, 10, 50, and 100 μM. The excised trifoliates treated with paraquat were placed in a growth chamber with 25 ± 5 °C temperature and continuous light of 600 μE m^{-2} s^{-1} PPFD for 12 h. Chlorophyll content was determined spectrophotometrically according to the method of Arnon [15], following extraction of the excised trifoliates with dimethyl sulfoxide (DMSO) [16]. Each treatment was replicated two times and the experiment was repeated in time.

Paraquat effects on CO_2 fixation by isolated leaf cells of Kwangkyo and Hood soybean

Trifoliate leaves from seedlings of both cultivars were detached from plants, rinsed with distilled water, had their midribs removed, and were cut into small 1 mm × 1 cm strips with a sharp razor blade. Two grams of cut leaf tissue were infiltrated under vacuum with the enzyme macerase and maceration of the tissue was facilitated through slow magnetic stirring and repeated washings of the released cells through centrifugation. Detailed descriptions of these procedures have been given in earlier publications [17, 18]. The released cells were diluted up to the desired volume with an incubation medium containing 0.2 M sorbitol, 2 mM $Mg(NO_3)_2$, 1 mM $CaCl_2$ and 50 mM HEPES-KOH (pH 7.8). Chlorophyll content was determined by the method of Arnon [15]. CO_2 fixation was assayed in 25 ml Erlenmeyer flasks containing 2 ml of the cell preparation, 0.1 ml of 5 mM $NaH^{14}CO_3$ (sp. act. 57.3 mCi/mmol) containing 2 μCi of radioactivity and 0.05 ml of paraquat solutions (0, 5, 10, 50, and 100 μM). Each treatment was replicated three times. Samples were collected at 1, 2, 4, and 6 h after the initiation of the experiment and the effect of paraquat on CO_2 fixation was calculated as μmol of $^{14}CO_2$ fixed per mg of chlorophyll.

Paraquat effects on Kwangkyo and Hood soybean chlorophyll fluorescence induction

The intensity of chlorophyll fluorescence from excised leaf tissue was determined with a portable fluorometer (Model SF-10, Richard Brackner Research, Ltd., Ottawa, Canada) and an X-Y plotter (Model 70158, Hewlett Packard, San Diego, California) according to the method of Ahrens *et al.* [19]. The fluorometer was adjusted to emit light of 10 μE m^{-2} s^{-1} for 7 sec. Before each measurement the instrument was adjusted to zero with the sensor resting on a black cloth. Fluorescence was measured on the adaxial surface of the same spot in the center leaf of excised trifoliates from both soybean cultivars grown in vials containing either distilled water (control) or 100 μM paraquat (treated) at several time intervals after treatment (0, 30 min, 1, 2, and 3 h). Three replications of each treatment were used.

Paraquat effects on the growth of callus tissues from Kwangkyo and Hood soybean

Calli were derived from soybean leaves on a Murashige and Skoog medium supplemented with 2 ppm of 2,4-D and with 1% agar (w/v). Calli of the same size were transferred to petri dishes containing the same medium and various concentrations of paraquat (0, 0.1, 1, 10, 100, and 1000 μM). Petri dishes were placed in a dark incubator with 25 °C, and callus growth was measured at 30 days after inoculation. Each petri dish had five calli, and each treatment was replicated as least two times.

Results and Discussion

Paraquat effects on seedlings of "Kwangkyo" and "Hood" soybean

Data in Table I present visible injury and desiccation levels observed in seedlings of Kwangkyo and Hood soybean, 24 h after exposure to paraquat. At the high concentration of 500 and 1000 μM, paraquat caused greater visible injury to seedlings of Hood soybean than those of Kwangkyo. The corresponding desiccation levels of the same seedlings confirmed that treatment with 500 and 1000 μM of paraquat caused greater injury to Hood than to Kwangkyo soybean (Table I). These results illustrate the rapid herbicidal action of paraquat which is caused by the paraquat-mediated generation of toxic oxygen species that peroxidize membrane lipids and desiccate green tissues under conditions of strong light [1, 2]. Visible injury and desiccation levels of plant tissues treated with herbicides causing oxidative stresses have been used by Cullner *et al.* [13] to demonstrate the differential response of resistant and susceptible cultivars of tobacco to the herbicides paraquat and acifluorfen.

Table I. Visible injury and desiccation of Kwangkyo and Hood soybean seedlings after exposure to paraquat at 600 μE m^{-2} s^{-1} PPFD for 24 h.

Paraquat [μM]	Visible injury[a]		Desiccation [%][b,c]	
	Kwangkyo	Hood	Kwangkyo	Hood
0	–	–	21 a	20 a
10	–	–	21 a	20 a
50	*	**	22 a	20 a
100	**	**	22 a	21 a
500	**	***	25 b	30 b
1000	***	****	33 c	45 c

[a] Visible injury symptoms: –, no injury; *, weak necrosis along major veins; **, necrosis along major veins; ***, approximately 50% of leaf surface is yellow or brown; ****, total surface is brown, desiccation.

[b] Desiccation level (%): the dry shoot and leaf weight as percentage of the fresh shoot and leaf weight, determined after herbicide treatment (see ref. [14]).

[c] Means within columns followed by the same letter are not significantly different at the 0.05 level by the LSD test.

Paraquat effects on chlorophyll content of excised trifoliates of Kwangkyo and Hood

Fig. 1 shows the chlorophyll content of excised first trifoliate leaves from both soybean cultivars exposed to paraquat for 12 h under greenhouse conditions. The chlorophyll content of control trifoliates was similar in both cultivars of soybean (3.0 mg Chl/ml in Kwangkyo and 3.2 mg Chl/ml in Hood). Paraquat applied at concentrations of 5 μM or greater caused a significant reduction of chlorophyll in Hood soybean (Fig. 1). In the tolerant Kwangkyo soybean, paraquat reduced chlorophyll when used at concentrations of 10 μM or greater (Fig. 1). However, the chlorophyll reduction caused by any concentration of paraquat on Hood soybean was about 2-fold greater than the chlorophyll reduction caused on Kwangkyo. The concentration of paraquat that caused a 50% reduction in the chlorophyll content of excised first trifoliates of Hood soybean was 5 μM, whereas about 50 μM of paraquat were needed to cause a similar degree of chlorophyll reduction in Kwangkyo soybean. These results indicate that there is an approximate 10-fold margin in the differential

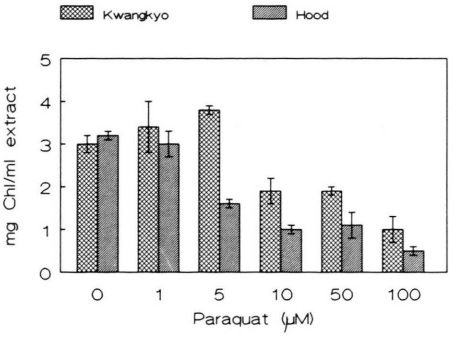

Fig. 1. The effect of paraquat on chlorophyll content of excised first trifoliates from Kwangkyo and Hood soybean at 12 h after treatment.

response of Kwangkyo and Hood soybean to the herbicide paraquat.

Paraquat effects on CO_2 fixation by isolated leaf cells of Kwangkyo and Hood

The effects of paraquat on $^{14}CO_2$ fixation by enzymatically isolated leaf mesophyll cells of both soybean cultivars after 1 and 4 h of incubation are presented in Table II. Treatment with all concentrations of paraquat (5, 10, 50, and 100 μM) caused a significant inhibition (50–60%) in the fixation of CO_2 by isolated cells of Kwangkyo soybean after 1 h of incubation with $NaH^{14}CO_3$. At the same incubation time, paraquat inhibited also the CO_2 fixation by isolated cells of Hood soybean, but to a lesser degree (14–50% inhibition). As the incubation time increased, the paraquat-mediated inhibition rates of CO_2 fixation by isolated leaf cells of both soybean cultivars increased and at incubation times of 4 h or greater, the inhibition caused by all concentrations of paraquat on CO_2 fixation by isolated cells of both cultivars was very similar (Table II).

It is obvious, that at the cell level paraquat is equally effective in interfering with the ability of both soybean cultivars to photosynthetically fix carbon dioxide. The absence of any differential effects of paraquat on the photosynthetic capacity of cells or chloroplasts isolated from resistant and susceptible biotypes of hairy fleabane or *Conyza* [*Conyza bonariensis* (L.) Cronq.] have been reported by Fuerst *et al.* [5]. Use of isolated leaf cells eliminates any barriers that may be involved in the absorption and/or movement of paraquat when it is applied to soybean seedlings or to excised trifoliate leaves. These results point out that differential absorption and/or translocation may be involved in the observed differential response of Kwangkyo and Hood soybean to the herbicide paraquat.

Paraquat effects on Kwangkyo and Hood soybean chlorophyll fluorescence induction

Chlorophyll fluorescence induction was measured in excised first trifoliate leaves from the two soybean cultivars at several time intervals following treatment with 100 μM of paraquat. These results are shown in Fig. 2. The chlorophyll fluorescence induction tracings from non-treated excised first trifoliates of Kwangkyo and Hood soybean were very similar. As early as 30 min after paraquat application there was a rapid penetration of the herbicide into excised first trifoliate leaves, indicated by the suppression of the variable fluorescence (F_v) in both cultivars (Fig. 2). However, the suppression of variable fluorescence by paraquat was much greater in leaves of the susceptible Hood cultivar, reaching maximum levels as early as 30 min. In leaves of the tolerant Kwangkyo cultivar, the paraquat-induced suppression of variable fluorescence was limited at 30 min and 2 h after treatment (Fig. 2). At 3 h (Fig. 2) and at greater time periods (data not shown) the levels of suppression of variable fluorescence by paraquat were similar in both cultivars. Fluorescence induction measurements following paraquat application

Table II. The effect of paraquat on $^{14}CO_2$ fixation of enzymatically isolated mesophyll cells of Kwangkyo and Hood soybean.

Incubation time [h]	Paraquat [μM]	Kwangkyo $^{14}CO_2$-fixed [μmol/mg Chl][a]	Kwangkyo Inhibition [%]	Hood $^{14}CO_2$-fixed [μmol/mg Chl]	Hood Inhibition [%]
1	0	23 ± 0.1	0	23 ± 0.1	0
	5	9 ± 0.2	62	15 ± 0.1	36
	10	11 ± 0.2	52	14 ± 0.2	40
	50	9 ± 0.1	64	20 ± 0.5	14
	100	9 ± 0.1	62	12 ± 0.1	50
4	0	65 ± 0.7	0	47 ± 0.1	0
	5	10 ± 0.1	85	12 ± 0.1	73
	10	12 ± 0.1	82	12 ± 0.1	74
	50	8 ± 0.1	88	13 ± 0.1	72
	100	8 ± 0.1	89	10 ± 0.1	79

[a] Values represent means of three replications ± standard error.

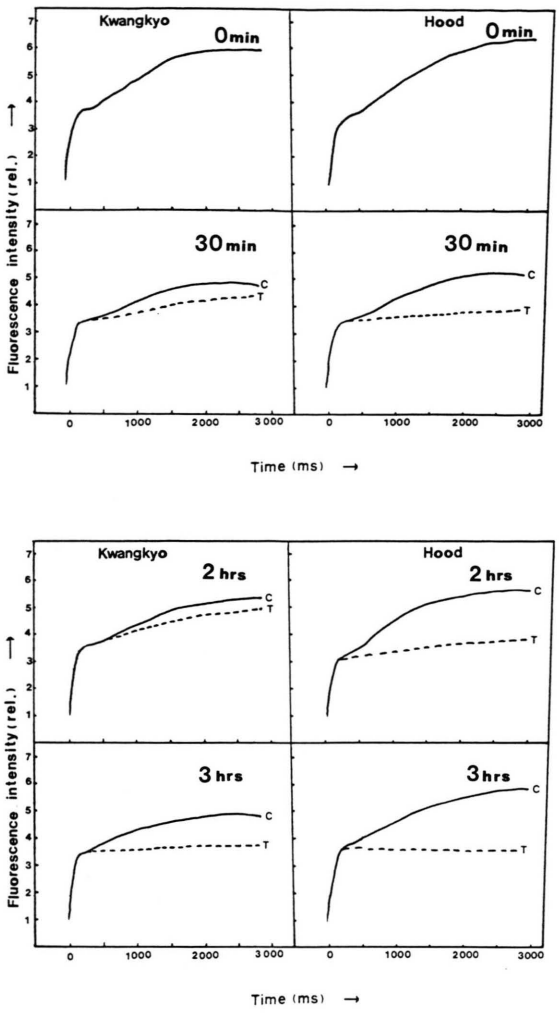

Fig. 2. Chlorophyll fluorescence induction tracings from first trifoliates of Kwangkyo (left) and Hood (right) soybean at various time intervals after treatment with 100 μM paraquat. For each time period after treatment, a control (C) and a paraquat-treated (T) induction tracing is included.

have been used previously by other researchers to demonstrate paraquat penetration into leaf tissues or chloroplasts isolated from weed biotypes resistant or susceptible to this herbicide [1, 20].

The observed differential effects of paraquat on chlorophyll fluorescence induction in excised trifoliates of Kwangkyo and Hood soybean appear to support further the potential contribution of differential translocation or sequestration in the tolerance of Kwangkyo soybean to paraquat.

Paraquat effects on the growth of callus tissues from Kwangkyo and Hood soybean

The effects of paraquat on the growth of callus tissues derived from leaves of both soybean cultivars are shown in Fig. 3. At concentrations of 1 μM or greater, paraquat reduced significantly the growth of calli derived from both cultivars. The inhibitory effect exerted by some concentrations of paraquat was more pronounced on the growth of Hood calli rather than the growth of Kwangkyo calli. At 10 μM, for example, the inhibitory effect of paraquat was 4 times greater on the growth of Hood calli than that of Kwangkyo calli (Fig. 3).

The observed inhibitory effects of paraquat on the growth of these non-green, heterotrophic callus tissues of soybean illustrates further the lack of the involvement of photosynthesis in the differential response of Kwangkyo and Hood soybean to this herbicide. These effects of paraquat on the growth of these callus tissues are most likely due to secondary actions caused by paraquat rather than its primary effects on photosynthesis and chlorophyll synthesis.

In summary, the comparative effects of paraquat on visible injury, desiccation levels, chlorophyll content, CO_2 fixation and chlorophyll fluorescence induction were examined using either whole seedlings or excised trifoliates, isolated leaf cells and callus tissues of Kwangkyo and Hood soybean. The obtained results confirmed the differential response of Kwangkyo and Hood soybean to the herbicide paraquat, reported initially by Kim *et al.* [12]. The margin of the observed dif-

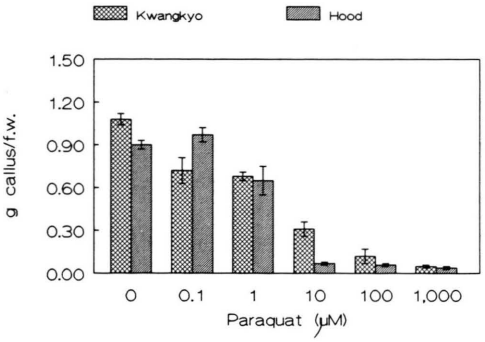

Fig. 3. The effect of paraquat on the growth of dark-grown callus tissues derived from leaves of Kwangkyo and Hood soybean at 30 days after inoculation.

ferential response of Kwangkyo and Hood soybean to paraquat is narrow (about 10-fold) and considerably smaller than the 100-fold or greater margins reported for the differential response of resistant and susceptible biotypes of several weeds such as *Conyza* sp. to this herbicide [1].

Taken as a whole, the obtained results support the potential involvement of differential absorption and/or translocation as a mechanism explaining the differential response of Kwangkyo and Hood soybean to the herbicide paraquat. Slow movement or a delay in the movement of paraquat into the mesophyll leaf tissues of Kwangkyo soybean may explain the limited tolerance of this soybean cultivar to this herbicide. The results of our comparative studies on the absorption, translocation, and metabolism of radiolabeled paraquat in Kwangkyo and Hood soybean have been reported elsewhere [21]. Nevertheless, additional mechanisms such as enhanced levels and/or activities of antioxidant enzymes may also contribute to the observed tolerance of Kwangkyo soybean to paraquat. The comparative effects of paraquat on antioxidant components and scavenging enzymes in Kwangkyo and Hood soybean are discussed in the next paper.

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