Indigenous Paraquat Resistance in Korean Foxglove (*Rehmannia glutinosa* Libosch.) and In Vivo Multiple Adventitious Buds Formation

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Abstract

Oriental or Korean foxglove (*Rehmannia glutinosa* Libosch., Fam. Scrophulariaceae) is used in a wide variety of medicinal drug applications in Korea, China and Japan. In traditional medicine, both the fresh and dry roots are used differentially in the treatment of wounds and bruises, as a poison antidote, and as a blood coagulant. Rice-wine cooked foxglove is used to improve the blood, as an overall tonic to improve health and vigor, in the treatment of diabetes and hypertension. Three types are used in traditional oriental medicine, *Rehmannia glutinosa,* native to Korea and another indigenous to Japan, and *R. glutinosa* var. *hueichingensis,* native to China. The foxgloves differ not only in their origin, but also in their anatomy and application. Korean foxglove is cultivated in the Republic of Korea and weed control is an expensive agricultural input by the small farmer. By chance, a Korean farmer observed that the foxglove plants under cultivation were not visually damaged following an application of paraquat, a potent non-selective herbicide. As a consequence, our research group was invited to confirm whether this population of Korean foxglove was 'resistant' to paraquat, and whether further selection is possible to develop a paraquat resistant Korean foxglove. Here, two populations, Korean foxglove, the purported resistant line, and a commercial Chinese foxglove variety, and observed paraquat sensitive variety, were greenhouse grown, and subjected to foliar application of paraquat over a wide concentration range (1x, 2x and 4x). The Korean foxglove population exhibited little damage, all plants appeared to survive the herbicide application two days after treatment, and continued to re-grow through the entire recovery period. Final survivability of the Korean foxglove was >83%, and final root growth and weight were greater in the paraquat treated plants than its untreated controls. In contrast, Chinese foxglove was sensitive at all paraquat concentrations, resulting in a complete die-back of all foliage two days after treatment yet 50-66.7% of plants survived the herbicide treatment. Those surviving plants exhibited reduced plant growth and root yield relative to their untreated controls. Following exposure to paraquat, we also observed in vivo multiple adventitious shoots formed (from 4.2% to 20.8%, from 1x to 4x rate) in the Korean foxglove, but no adventitious shoots were seen in the Chinese foxglove. Our results suggest that indigenous Korean foxglove is tolerant to paraquat, that clonal selection can be made for further selection to develop a paraquat resistant line. The mechanism of tolerance is unknown.

INTRODUCTION

Korean Foxglove (Fam. Scrophulariaceae) is a popular traditional oriental medicinal herb widely used in Korea, China and Japan for a variety of medical applications including in the treatment of wounds and bruises, as a poison antidote, and as a blood coagulant. Rice-wine cooked foxglove is used to improve the blood, as an overall tonic to improve health and vigor, and in the treatment of diabetes and hypertension.

Commonly referred collectively as Korean foxglove, this species in the trade appears to reflect three types of foxgloves: Oriental or Korean foxglove (*Rehmannia glutinosa* Libosch., native to Korea, a second type indigenous to Japan, and lastly, *R. glutinosa* var. *hueichingensis*, native to China. The fresh or dried roots, both used differently, represent the product of commerce. The roots appear to vary by origin or source. Chinese foxglove has very large and thick roots, the roots of Japanese foxglove are thin and long and the roots of Korean foxglove are intermediate. Korean foxglove roots contain a wide range of natural products including β-sitosterol, catalpol, D-fructose, D-galactose, manninotriose, rehmanniosides, and verbascose (Mun et al. 1984).

Korean foxglove is cultivated in the Republic of Korea by small farmers, and weed control represents one of the highest agricultural inputs. Consequently there has been interest in developing both chemical and non-chemical approaches to reduce the costs and time involved in weeding. Paraquat, well known as a strong non-selective herbicide was inadvertently sprayed during commercial application to Korean foxglove, and farmers observed little damage. This study builds upon a larger program to develop a paraquat resistant foxglove and related programs probing the mechanism(s) of resistance. The objectives of this study presented were to confirm whether this population of Korean foxglove was 'resistant' to paraquat, and whether further selection is possible to develop a paraquat resistant Korean foxglove. Two populations, Korean foxglove, the purported resistant line, and a commercial Chinese foxglove variety grown in Korea, an observed paraquat sensitive variety, were greenhouse grown, subjected to foliar paraquat applications and observed for injury.

MATERIALS AND METHODS

In this initial study, two foxgloves were comparatively evaluated. Rootstock of Korean foxglove (*Rehmannia glutinosa* Libosch) var. 'Koryo' (known in Korean as Jiwhang), and a Chinese foxglove (*Rehmannia glutinosa* var. *hueichingensis)* var. 'Jiwhang No.1' (also known in Korean as Jiwhang) were obtained from the National Crop Experiment Station, Rural Development Association, Suwon, Kyunggi, Republic of South Korea, and brought over to Rutgers University for this work.

All trials were conducted from July 2001 through March 2002, under controlled greenhouse conditions using the Plant Biology greenhouses, Cook College. Rootstock was transferred on July 19 2001 into 12 cm diameter plastic pots using a potting medium (Pro-Mix BX, Premier Company, Quakertown, PA, USA) and grown in a greenhouse supplemented with a 16 h photoperiod of 85mmol m^{-2} s^{-1}. After nine months of growth, the foxglove was ready for experimentation. All plants were physically taken outside adjacent to the greenhouse for treatment to paraquat dichloride using the commercial product Gramoxone (Zeneca). The herbicide was applied to 24 individual plants at each of three different concentrations (1x, 2x, and 4x) with 1x serving as the recommended standard rate of 2.605ml product/L. Control plants (also 24 individual plants from each of the two varieties) were sprayed only with deionized water, also outside at the same time and with the same applicator system. Plant damage was assessed at three time intervals following treatment (2, 5 and 40 days after paraquat applications), and plants were assessed for foliar damage, survival, and at 40 days after treatment (DAT) for plant height, leaf number, leaf width, root length and dry root weight. Dry weight was obtained by placing the fresh roots into a low temperature forced air dryer at 38C.

RESULTS

Gramoxone at three concentrations was applied to healthy Korean and Chinese foxglove. Two days following treatment, all leaves of the Chinese foxglove were burnt, and the green foliage was largely necrotic (Table 1). Only 12.5% of the 24 treated plants survived at the lowest (1x) paraquat treated rate. All plants from the Chinese foxglove appeared to be dead by Day 2 at the higher herbicide concentrations (2x and 4x) as assessed by the complete kill of the top growth of the plant. As expected, none of the control plants of the Chinese foxglove exhibited any damage. In contrast, plants from the

Korean foxglove lines survived and exhibited a high degree of tolerance to the paraquat treatment with little to no die-back at any of the herbicide applications, though leaf damage was noted at all herbicide applications from day 2 onward. varying range of visual damage (Table 1). With the recommended application rate (1x), only about 0.6±0.7 of the leaves of the Korean foxglove exhibited foliar injury. The extent of foliar injury in the Korean foxglove did increase over time and with increased herbicide application rate.

Chinese foxglove did recover over time, despite initial complete loss of foliage. Young shoots developed from the inside meristem so that by 5 day following treatment, new growth was visible and by the final 40 DAT, ca. 50% of the plants had survived and were re-growing. Korean foxglove exhibited a higher survival rate and improved growth following the application or paraquat.

At 40 DAT, plant height, root length and root dry weight was greater to or equal to its control plants (Fig. 1). No differences in number of leaves or leaf width were noted (Table 2). In contrast, plant height, root length and root dry weight was reduced in Chinese foxglove plants treated with paraquat relative to its control plants. Comparing overall plant growth, it was clear that the Chinese foxglove is a larger more robust plant, being both taller in height, and with a known larger and thicker root. Chinese foxglove (cv. 'Jiwhang 1') root weight reached almost four times that of the Korean foxglove (cv. 'Koryo'), respectively (Table 2). Under greenhouse conditions, the Chinese foxglove exhibited much more flowering but no seeds developed by either type for collection. The plant is normally vegetative propagated by rootstock cuttings and under commercial practices, the Korean farmers remove the floral buds and flowers to encourage root growth.

At 40 DAT, multiple adventitious shoots from the paraquat treated Korean foxglove plants were observed, while none were found in the Chinese foxgloves. In vivo multiple adventitious shoots formed at 4.2%, 8.3% and 20% from the paraquat-treated plants at the 1x, 2x, and 4x rates, respectively (Table 3, Fig. 2).

CONCLUSIONS

Two populations of Korean foxglove, a purported resistant line, and a commercial Chinese foxglove, an observed paraquat sensitive variety, were greenhouse grown and subjected to foliar paraquat applications at three concentration ranges (1x, 2x and 4x). Korean foxglove population exhibited little damage and all plants appeared to survive the herbicide application 2 days after treatment, and continued to re-grow through the entire recovery period. Final survivability of the Korean foxglove was >83%, and final root growth (length and weight) was greater in the paraquat treated plants than its untreated control. In contrast, Chinese foxglove was sensitive at all paraquat applications, resulting in the complete die-back of all foliage two days after treatment yet plants did survive and there was a total of 50-66.7% (paraquat concentration dependent) of the plants that survived the herbicide treatment. Those surviving plants exhibited reduced plant growth and root yield relative to their untreated controls. Following exposure to paraquat, we also observed in vivo multiple adventitious shoots formed (from 4.2% to 20.8%, from 1x to 4x rate) in the Korean foxglove, but no adventitious shoots were seen with Chinese foxglove. Our results suggest that indigenous Korean foxglove is tolerant to paraquat, that clonal selection can be made for further selection to develop a paraquat resistant line. The mechanism of tolerance is unknown, but the use of this plant as a source of paraquat resistance gene(s) may be promising to examine.

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Tables

Table 1. Plant damage, survival of top growth and growth recovery of Korean and Chinese foxglove subjected to three concentrations of paraquat.

DAT[1] and Paraquat Application rate[2]	Korean Foxglove			Chinese Foxglove		
	% of leaf showing injury	Leaf no/pl	Survival of top growth (%)	% of leaf showing injury	Leaf no/pl	Survival of top growth (%)
2Days-1X	0.6±0.7	8.4±1.4	100	---[3]	---	12.5
2X	1.7±1.1	8.3±1.2	100	---	---	0
4X	7.6±1.2	8.6±1.4	100	---	---	0
5Days-1X	1.2±1.1	9.6±1.6	100	---	---	37.5
2X	4.7±2.2	9.4±1.6	100	---	---	16.7
4X	8.9±0.9	9.3±0.4	91.7	---	---	8.3
40Days-1X			87.5			58.3
2X			87.5			66.7
4X			83.3			50.0

[1]= days of treatment.
[2]1x=2.605 ml product/L.
[3]=all leaves were dead, measurements not possible.

Table 2. Plant growth and root yield following paraquat applications to Korean and Chinese Foxglove.

Paraquat application[1]		Plants ht (cm)	No. of leaves	Leaf width (cm)	Root length (cm)	Root weight (g)
Korean foxglove	Control	15.2±1.8	12.7±1.3	4.3±0.5	12.5±1.1	34.8±11.4
	1X	15.1±1.7	12.3±1.2	4.2±0.6	13.1±0.9	38.6±15.2
	2X	13.3±2.5	10.8±1.3	3.9±0.6	13.2±4.1	32.4±17.7
	4X	17.3±1.7	13.5±1.1	5.2±0.8	13.4±1.4	33.9±17.5
Chinese foxglove	Control	22.5±4.1	11.3±1.6	5.7±1.2	14.4±1.0	122.6±31.9
	1X	20.3±2.9	10.7±2.0	5.8±1.9	12.6±0.8	108.7±29.7
	2X	20.7±3.3	10.9±2.3	5.5±1.0	13.2±1.5	107.6±37.2
	4X	23.5±3.6	13.0±2.8	6.2±1.4	14.3±1.7	107.9±24.6

[1]1x=2.605 ml product/L.

Table 3. Multiple adventitious buds formation following paraquat application to Korean and Chinese foxglove.

Paraquat application[1]		Multiple adventitious shoot formation (No.)	Multiple adventitious shoot formation (%)
Korean foxglove	Control	0	-
	1X	1	4.2
	2X	2	8.3
	4X	5	20.8
Chinese foxglove	Control	0	0
	1X	0	0
	2X	0	0
	4X	0	0

[1]1x=2.605 ml product/L.

Figures

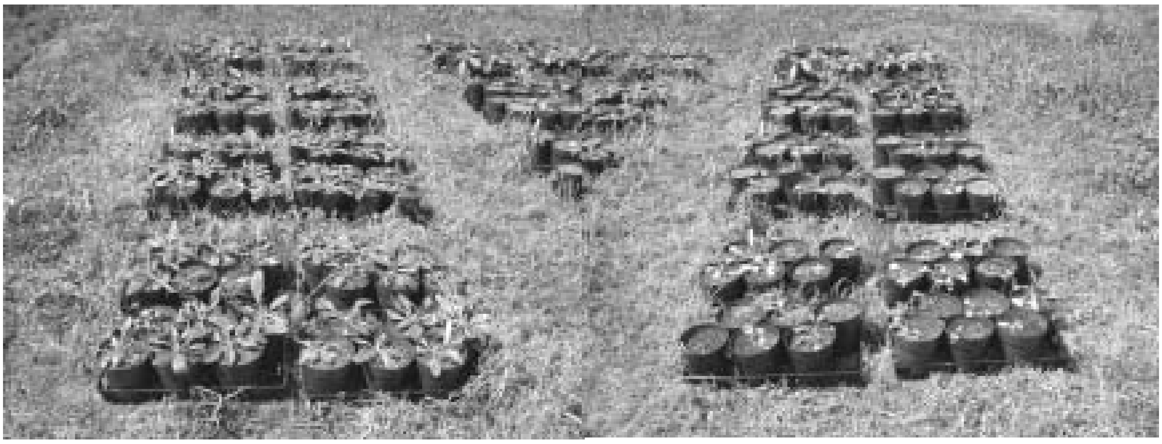

Fig. 1. Paraquat 'resistant' Korean foxglove variety "Koryo"(Left) and Chinese foxglove nonresistant variety "Jiwhang1"(Right). Photo taken two days after herbicide treatment.

Fig. 2. Adventitious shoot formation from Korean foxglove variety "Koryo in greenhouse pot culture subjected to a 4x paraquat application.