Difference in Growth, Yield and Isoflavone Content among Soybean Cultivars under Drained Paddy Field Condition

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ABSTRACT : Naturally occurring soybean isoflavones are known to be influenced by various genetic and environmental conditions. Growth, yield, and isoflavone content were determined in four different cultivars of soybean grown under drained paddy and upland fields. Most of growth characteristics and yield components of four different soybean cultivars harvested in drained paddy field were greater than those in upland field, regardless of cultivar. By means of high performance liquid chromatography, total daidzein and genistein contents of soybean in drained paddy field were increased up to 40 and 35%, respectively, compared with those in drained paddy field. Besides isoflavone contents, the growth and yield of soybean were significantly affected by cultivar and field conditions, indicating the necessity of genetic program for soybean cultivars appropriate to drained paddy field conditions. In conclusion, converting paddy field into upland may effectively improve soybean cropping system, especially in terms of isoflavone increment under paddy field conditions.

Keywords: isoflavones, drained paddy fields, soybean, yield components

Soybeans and soy foods have been consumed abundantly, especially by Koreans, as a main dietary source of protein and fat (Kwon & Song, 1996). Several studies attributed the physiologically active properties of soybeans to not only protein but isoflavones which are mainly present in the conjugated forms in the whole seed (Wang & Murphy, 1994 a and b). The most common isoflavones in soybeans are genistein, daizein and glycitein, which occur as aglycone, glucoside acetylglucoside or malonyglucoside (Wang & Murphy, 1994a). Isoflavones including genistein, daidzein, and glycitein have been reported to have anticarcinogenic, anti-fungal, and antioxidant activities (Kim, 1996; Coward *et al.*, 1993; Lee *et al.*, 1991; Barnes, 1995; Naim *et al.*, 1974). In particular, genistein, which is a potent tyrosine kinase inhibitor, has attracted much attention due to its possible role in preventing breast and prostate cancers (Barnes, 1995; Record *et al.*, 1995; Wei *et al.*, 1995; Akiyama *et al.*, 1987). Another study (Kim *et al.*, 1996) also suggested that genistein might be a promising chemopreventive agent due to its capability to modulate cellular detoxification enzyme (s). Daidzein, the other main isoflavone present in soybean, was also in association with inhibition of human breast cancer cells and retain antiestrogenic activity (Coward *et al.*, 1993; Peterson & Barnes, 1991).

The content of isoflavones in soybeans is quite variable by genetic and environmental conditions such as growing location, temperature during seed fill (Dixon *et al.*, 1995; Lee *et al.*, 2003). Growth conditions including temperature and precipitation were reported to have a great effect on the isoflavone content of mature soybean seeds (Wang & Murphy, 1994b; *et al.*, 1995). They suggested the isoflavone contents of soybeans grown at high temperature be significantly lowered. Tsukamoto *et al.* (1995) reported a wide variation in total isoflavone content depending on varieties, sowing dates, weather and locations. For instance, total isoflavone content of the same variety was 5.8 times different in response to different sowing dates, with 246 versus 1,423 mg kg^{-1}. This difference was postulated to be associated with weather conditions such as temperatures during the maturation of soybean seed.

Identification and quantification of isoflavones have been performed by several methods including high performance liquid chromatography (HPLC) (Wang & Murphy, 1994 a and b; Wang *et al.*, 1990; Eldridge & Kwolek, 1983), gas-liquid chromatography (Naim *et al.*, 1974), isotope dilution gas chromatography-mass spectrometry (Dwyer *et al.*, 1994), and thin layer chromatography (Kim *et al.*, 1994).

In Korea, soybeans are traditionally grown in upland fields but are now also grown in drained-paddy fields after converting paddy fields into upland fields for cultivation of soybean and other crops. Isoflavone content and yield as important determinants of soybean quality are believed to be variable according to growing conditions. Therefore, this study was conducted to determine the change in growth, yield and isoflavone content of soybeans between the two different growing conditions, drained-paddy and upland fields. This research will provide key information for the

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development of functionality and productivity in soybean cropping system.

MATERIALS AND METHODS

Culture conditions

Soybean seeds of cultivars, 'Eunha', 'Pungsan', 'Saeol' and 'Taegwang' were planted on June, 2002, in the private farmland of Jangheung, Jeonnam province. Organic matter (%), pH (1:5), and P_2O_5 (mg kg^{-1}) of paddy/upland fields investigated were 2.9/2.8, 6.1/6.4 and 489/636, respectively. Climatic data for the period of June to October in 2002 in cultivated field were summarized in Table 1.

Growth and yield components of soybean in drained paddy and upland fields

Soybean samples were harvested at 18 weeks after sowing for measurement of crop growth. Lodging degree by visual rating, stem length, number of branch and number of nodule were measured in plants collected from three parts of each field.

For the measurement of soybean yield, major yield components such as number of pods, number of grains per pod, 100-seed weight, and grain weight were measured in plants collected from three parts of each field at 18 weeks after seeding. When the F-test was significant ($P<0.05$), means among cultivars were separated on the based on the least significant difference (LSD) at the 0.05 probability level (SAS Institute, 1998).

Quantitative determination of isoflavones

HPLC-grade solvents were purchased from Merck (Rathway, NJ, USA). Genistein, glycitein and daidzein standards were purchased from Sigma (St. Louis, MO, USA), Plantech (Reading, UK) and Indofine Chemical (Somerville, NJ, USA) respectively. Other reagent grade chemicals were obtained from Sigma. HPLC column (3.9 mm × 30 cm length), Waters Xterra RP18 (5 μm), was from Waters Associates (Milford, MA, USA).

Soybean seeds were powdered using a mill to pass through a 1-mm screen. The portion of soybean flour was allotted for the proximate analysis. Five grams of dry ground sample suspended in 15 ml 1N-HCl were heated at 100 °C for 3 hr in a heating block. At the end of digestion, 15 ml of methanol was added to achieve the complete solvation of isoflavones. The mixture was settled for a few minutes, and the supernatant was filtered through Corning syringe filter (nylon, 0.2 μm) and used for HPLC analysis (Choi *et al.*, 1996; Yi *et al.*, 1997).

Analysis of isoflavones was performed according to the method described by So *et al.* (2001) with a slight modification. The 20 μ*l* filtrate was injected to HPLC equipped with the column after the system had been equilibrated at ambient temperature and stabilized the UV detector with mobile phase (Acetonitrile : Water = 35 : 65) at a flow rate of 1 ml/min for 30 min. Eluant was detected at 254 nm and chromatogram was recorded for 20 min. Isoflavones were identified by retention time and internal addition of standards, and their contents and recoveries were calculated by comparing peak area with those of standards subjected to same treatments.

RESULTS AND DISCUSSION

Soybean growth in drained paddy and upland Fields

Degree of lodging, stem length, number of branch and number of nodule in drained paddy field were significantly greater than those in upland fields. Logging degree and number of branch of soybean in drained paddy field increased by 70% and 11%, respectively, compared to those in upland fields. However, number of nodule of soybeans in paddy field was lower than those in upland fields. On the other hand, stem length of soybeans was not affected by field conditions. A significant difference in growth characteristics among cultivars was observed (Table 2).

Table 1. Climatic data at Jangheung region for the period of June to October in 2002.

Month	Air temperature (°C)			Sunshine duration (hr/month)	Precipitation (mm/month)
	Mean	Max	Min		
June	20.9	26.7	15.5	269.0	122.5
July	23.7	27.7	21.0	186.2	336.5
Aug.	23.7	27.7	20.9	173.9	809.0
Sept.	19.7	26.4	14.7	236.7	115.0
Oct.	13.1	20.4	6.8	222.8	46.0

Table 2. Growth characteristics of 4 soybean cultivars grown in drained paddy and upland fields.

Cultivar	Growth characteristics							
	Lodging degree (0 - 9)†		Culm length (cm)		No. of branches		No. of nodules	
	Paddy	Upland	Paddy	Upland	Paddy	Upland	Paddy	Upland
Eunha	3.7 b‡	1.3 b	56 a	57 a	4.5 a	3.8 a	30.1 b	26.3 b
Pungsan	6.3 a	5.3 a	49 b	47 b	4.5 a	4.2 a	45.9 a	52.1 a
Saeol	4.2 b	1.7 b	59 a	55 a	4.6 a	4.0 a	30.4 b	51.3 a
Taegwang	4.3 b	2.3 b	36 c	37 c	3.2 b	3.2 b	27.7 b	44.9 a
Mean	4.6 (170)#	2.7 (100)	50.0 (102)	49.0 (100)	4.2 (111)	3.8 (100)	33.5 (77)	43.7 (100)

†Lodging degree: 0 = no damage, 9 = severe damage.
‡Means within a field condition are not significantly different ($p<0.05$) from those with the same letter.
#Parentheses represent percent of soybean data from upland field.

Table 3. Yield components of 4 soybean cultivars grown in drained paddy and upland fields.

Cultivar	Yield components							
	No. of pod per plant		No. of grain per pod		100-seeds weight (g)		Yield (kg/10a)	
	Paddy	Upland	Paddy	Upland	Paddy	Upland	Paddy	Upland
Eunha	48.0 b†	40.3 b	2.7 a	2.8 a	12.9 c	11.3 c	255.0 a	214.0 a
Pungsan	70.0 a	64.7 a	2.4 b	2.4 b	11.3 d	8.5 d	271.0 a	171.1 b
Saeol	30.3 c	25.3 c	2.4 b	2.3 b	26.7 a	25.0 a	178.3 b	166.3 b
Taegwang	44.7 b	35.7 b	1.9 c	1.8 c	20.7 b	18.4 b	190.0 b	157.3 b
Mean	48.3 (116)‡	41.5 (100)	2.4 (104)	2.3 (100)	17.9 (113)	15.8 (100)	223.6 (126)	177.2 (100)

†Means within a field condition are not significantly different ($p<0.05$) from those with the same letter.
‡Parentheses represent percent of soybean data from upland field.

Yield components of soybean in drained paddy and upland fields

Yield components were significantly different in four different cultivars of soybean grown under drained paddy and upland fields. A significant difference in yield components among soybean cultivars was recognized. Drained paddy conditions increased number of pod per plant, 100-seed weight, and grain yield in paddy fields by 16, 4, 13, and 26%, respectively, compared with the upland field. Yield components of cultivars 'Eunha' and 'Pungsan' were greater than those of cultivars 'Saeol' and 'Taegwang' (Table 3). The results showed that cultivation in the paddy field conditions could increase soybean yields. Similar to earlier studies on upland-paddy rotation system (Kwon *et al.*, 1993; Ahn & Motomatsu, 1993), crop yields including Chinese cabbage, soybean, ginseng, cultivated in paddy field was increased more than in upland field

Quantitative determination of isoflavones

Total isoflavone contents were higher in two soybean cultivars 'Eunha' and 'Pungsan' from drained-paddy field than those of upland field, while total isoflavone contents were lower in 'Saeol' and 'Taegwang' (Table 4). Free genistein showed the highest level among isoflavones, and followed by daizein and glycitein. All the isoflavones were most abundantly present in cultivar 'Eunha' among cultivars tested regardless of cultivation method, while cultivar 'Saeol' contained the lowest amount. Total daidzein and genistein contents of soybean in paddy field were increased up to 40, 35% respectively, compared with those in upland field. Especially, total isoflavone contents in cultivars 'Eunha' and 'Pungsan' increased remarkably when the soybean was grown under paddy fields. However, no difference in glycitein contents of soybean between two cultivation methods was observed (Table 4). These results suggest that soybean isoflavone contents are influenced by cultivation method as well as genetic resource. The content of isoflavones in soybeans is quite variable by genetics and environmental conditions such as growing location, temperature during seed fill (Dixon *et al.*, 1995, Lee *et al.*, 2003). Cultivar 'Eunha' showed the highest levels in daidzein, genistein and glycitein regardless of cultivation condition, and fol-

Table 4. Isoflavone contents of 4 soybean cultivars grown in drained paddy and upland fields.

Cultivar	Isoflavone ($mg\ kg^{-1}$)							
	Daidzein		Genistein		Glycitein		Total	
	Paddy	Upland	Paddy	Upland	Paddy	Upland	Paddy	Upland
Eunha	740 a†	450 a	803 a	665 a	250 a	170 a	1794 a	1284 a
Pungsan	475 b	278 b	613 b	257 b	119 b	137 b	1207 b	671 b
Saeol	149 d	162 d	204 d	218 c	59 c	71 c	412 c	451 c
Taegwang	226 c	247 c	247 c	240 b	82 bc	128 b	555 c	615 b
Mean	398 (140)‡	284 (100)	467 (135)	345 (100)	128 (101)	127 (100)	992 (131)	755 (100)

†Means within a field condition are not significantly different ($p<0.05$) from those with the same letter.
‡Parentheses represents percent of soybean data from upland field.

lowed by 'Pungsan', 'Taegwang', and 'Saeol'. Total daidzein, genistein and glycitein contents in soybeans from drained-paddy field were 740, 803, and 250 mg/kg, respectively. The result indicates that isoflavone content could be influenced by cultivar as well as cultivation condition. Tsukamoto *et al.* (1995) have reported a wide variation in total isoflavone content depending on varieties, sowing dates, weather and locations.

We demonstrated that growth, yield and isoflavone contents in soybeans grown under drained paddy field were increased, compared with upland field, and that the variation in growth, yield and isoflavone content depended on variety. Soybeans produced under drained paddy field are expected to have maximum beneficial effect as biologically-active substances. Naturally occurring soybean isoflavones including genistein, daidzein, and glycitein have been documented to have anticarcinogenic, anti-fungal, and antioxidant activities in earlier studies. The content of isoflavones in soybeans was quite variable by genetics, especially cultivar. Growth conditions have a beneficial effect on the isoflavone content of mature soybean seeds. In Korea, now upland-paddy rotation system, converting paddy fields into upland fields for crop cultivation, are gradually increased national wide. However, we need further, more detail researches regarding effects of environmental conditions including water content, light condition, and soil properties, on isoflavone production of soybean.

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