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# Independent Inheritance between *df2* gene and *ti* gene in Soybean

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**ABSTRACT** Dwarfness and Kunitz trypsin inhibitor (KTI) protein in soybean is useful traits for basic studies. *df2* and *ti* gene control dwarfness and the expression of Kunitz trypsin inhibitor (KTI) protein in soybean, respectively. The objective of this research was to verify genetic linkage or independent inheritance of *df2* and *ti* loci in soybean. The $F_2$ population was made by cross combination between "Gaechuck#2" (*Df2Df2titi* genotype, KTI protein absence and a normal growth type) and T210 (*df2df2TiTi* genotype, a dwarf growth type and KTI protein present). A total of 258 $F_2$ seeds were analyzed for the segregation of KTI protein using SDS-PAGE. And so, 198 $F_2$ plants were recorded for the segregation of dwarfness. The segregation ratio of 3 : 1 for *Ti* locus (201 *Ti_* : 57 *titi*) and *Df2* locus (143 *Df2_* : 55 *df2df2*) was observed. Segregation ratio of 9 : 3 : 3 : 1 (116 *Ti_Df2_*: 44 *Ti_df2df2*: 27 *titiDf2_*: 11 *titidf2df2*) between *df2* gene and *ti* gene was observed ($\chi^2$= 3.53, P = 0.223). These results showed that df2 gene was inherited independently with the ti gene in soybean.

*Keywords* : *df2,* dwarf, Kunitz trypsin inhibitor, soybean, *ti*

**Soybean** [*Glycine max* (L.) Merrill] shows considerable diversity in stem height. Dwarf soybean plants have characteristics of short internodes and petioles. The leaves were about one-fourth normal size. In cultivar, "Habaro", dwarf plant was first found and *df* gene that controls dwarfness was assigned (Stewart, 1927; Woodworth, 1932). Dwarf plant found in "Habaro" cultivar was controlled by a single recessive gene, *df1*. From a colchicine-treated seedling of "Lincoln" cultivar, dwarf plant was found and was controlled by a single recessive gene, *df2* (Porter and Weiss, 1948). In cultivar "Adams", dwarf plants controlled by a single recessive gene, *df3* was found in the progeny of seeds irradiated with thermal neutrons (Byth and Weber, 1969). *df2* gene was inherited independently with *df3* gene (Byth and Weber, 1969). Fehr (1972) found dwarf plants controlled by a single recessive gene, *df4* in a row of the cultivar "Hark" and observed that *df4* gene was inherited independently with *df2* and *df3* gene. Weiss (1970) reported *df2* gene was located on classical linkage group 6 (molecular linkage group F, chromosome number 13).

The trypsin inhibitor proteins have been proposed as one of the major antinutritional factors (Westfall and Hauge, 1948). Bowman-Birk inhibitor and Kunitz trypsin inhibitor (KTI) have been reported to be present in soybeans (Kunitz, 1945). Much of the soybean trypsin inhibitor activity is thought to be due to the protein - A2 (Rackis *et al.*,1962), which generally is known as the Kunitz trypsin inhibitor. Soybean Kunitz trypsin inhibitor (KTI) is a small, monomeric and non-glycosylated protein containing 181 amino acid residues with 21.5 kDa (Kunitz, 1945). Several electrophoretic forms of KTI protein have been discovered. The genetic control of several forms, $Ti^a$ and $Ti^b$ (Singh *et al.*, 1969), $Ti^c$ (Hymowitz, 1973), $Ti^d$ (Zhao and Wang, 1992), $Ti^e$ (Wang *et al.*, 2001), and $Ti^f$ (Wang and Li, 2005) have been reported. These types are controlled by codominant multiple alleles at a single locus. Two soybean accessions (PI157440 and PI196168) lacking the KTI protein have been identified (Orf and Hymowitz, 1979). Orf and Hymowitz (1979) found that this form does not exhibit a KTI protein and is inherited as a recessive allele designated *ti*. The *Ti* locus has been located on linkage group 9 in the classical linkage map of soybean (Hildebrand *et al.*, 1980; Kiang, 1987), which is integrated in molecular linkage group A2 (chromosome number 8). Independent inheritance between *Le* gene and *Ti* gene was reported (Moraes *et al.*,2006; Orf

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and Hymowitz, 1979; Lee *et al.*, 2008). Also, Sung *et al.* (2010) reported that $Cgy_1$ gene was inherited independently with the *Ti* gene in soybean. Genetic relationship between morphological traits and seed components is very important to basic studies and breeding projects in soybean. The objective of this research was to verify the inheritance of *df2* gene and *ti* gene in soybean.

## MATERIALS AND METHODS

### Genetic materials

Two parents were used to develop $F_2$ population. "Gaechuck#2" parent has *ti* gene [Kunitz Trypsin Inhibitor (KTI) protein absent, *titi* genotype] and *Df2* gene (normal stem height, *Df2Df2* genotype). T210 parent has *df2* gene (dwarf, *df2df2* genotype) and *Ti* gene (KTI protein present, *TiTi* genotype). The seed of T210 parent was obtained from the USDA Soybean Germplasm Collection in Urbana, IL. The seeds of "Gaechuck#2" and T210 parents were planted to cross in a greenhouse in March 2009. The crosses of "Gaechuck#2" (*titiDf2Df2*) x T210 (*TiTidf2df2*) were made and $F_1$ seeds were obtained. $F_1$ seeds obtained were planted in a greenhouse in February 2010. $F_1$ hybridity was confirmed on morphological traits. $F_2$ seeds were harvested and were analyzed for the presence or absence of KTI protein. The seeds of parent and $F_2$ were planted at field in Jun 2010. Dwarfness of $F_2$ individual plants was recorded at R2 and R3 growth stage from field. Chi-square analysis was used to test the goodness-of-fit of observed ratios with expected ratios for independent assortment or linkage.

### Determination of Kunitz trypsin inhibitor protein

A piece of cotyledon from the parents and individual $F_2$ seed was removed to extract crude protein for determining the presence ( ` + ' ) or absence ('- ' ) of KTI protein electrophoretically and was incubated for 30 min (at room temperature) in 1 ㎖ Tris-HCl, pH 8.0, containing 1.56% v/v β-mercaptoethanol. After centrifugation, 50 ㎕ of the supernatant was added to an equivalent amount of 5X sample buffer [10% (w/v) sodium dodecyl sulfate (SDS), 50% (v/v) glycerol, 1.96% v/v β-mercaptoethanol, 1M Tris-HCl, pH 6.8]. The samples were boiled at 97℃ for 5 min and then centrifuged. Two microliters of the supernatant was loaded on a 12% acrylamide SDS polyacrylamide gel electrophoresis (SDS-PAGE) medium gels in Owl Separation Systems Inc (Model : P9DS, Portsmouth, NH USA). Electrophoresis was performed at 120 V for 7 hrs. Gels were stained overnight in an aqueous solution of 0.25 g Coomassie blue R250, 10% acetic acid, and 45% methanol. The gels were then destained with destaining solution (5% acetic acid, 14% methanol) for several hours. A Wide-Range SDS-PAGE molecular mass standard (Sigma Marker™, Product Code: M4038, St. Louis MO USA) containing 21.5 kDa (for KTI protein) was used to aid recognition of samples lacking the KTI protein.

## RESULTS AND DISCUSSION

A total of 258 $F_2$ seeds were obtained from the crosses of female parent, "Gaechuck#2" and male parent, T210 and was analyzed for the segregation of Kunitz trypsin inhibitor (KTI) protein. Part of the SDS-PAGE patterns of KTI protein that appeared in the parents and $F_2$ seeds from the cross was shown in Figure 1.

Bands for KTI protein were segregated in $F_2$ seeds. The segregation of $F_2$ seed for presence and absence of KTI protein is summarized in Table 1.

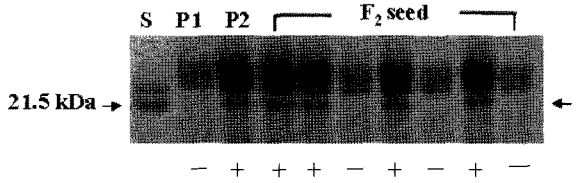


**Fig. 1.** Segregation of Kunitz trypsin inhibitor (KTI) protein in F2 seeds derived from the crosses between "Gaechuck#2" (*titiDf2Df2*) and T210 (*TiTidf2df2*). S: protein marker, P1: "Gaechuck#2", P2: T210. +: present of KTI protein, -: absent of KTI protein.

**Table 1.** Segregation of the presence or absence of the Kunitz trypsin inhibitor (KTI) protein in $F_2$ seeds from the crosses between "Gaechuck#2" and T210.

| KTI protein | Seed number | | χ2 (3:1) | P |
|---|---|---|---|---|
| | Observed | Expected | | |
| + | 201 | 193.5 | 1.16 | 0.281 |
| - | 57 | 64.5 | | |

+: presence, -: absence

Among 258 $F_2$ seeds, 201 $F_2$ seeds showed KTI protein and 57 $F_2$ seeds did not exhibit KTI protein. The segregation ration of the presence or absence of the KTI protein in the $F_2$ seeds well fitted to an expected 3:1 ratio ($\chi^2$ = 1.16, P= 0.281). This result shows that the absence of the KTI protein is controlled by a single recessive gene. Previous studies have shown that KTI protein is inherited by a single recessive allele (Orf and Hymowitz, 1979; Kim *et al.*, 2006, Lee *et al.*, 2008).

A total of 258 $F_2$ seeds obtained were deployed to advance $F_2$ plant generation. Only 198 $F_2$ seeds among them were germinated and plants were grown in field. The normal plant and dwarf plant was segregated in $F_2$ population (Fig. 2).

Among 198 $F_2$ plants, 143 $F_2$ plants showed the normal type and 55 $F_2$ plants showed dwarf type. The summarized data for the observed and expected distribution of $F_2$ plants are shown in Table 2. Segregation of the dwarf type gave a good fit to the 3 : 1 ratio based on $F_2$ phenotype (normal : dwarf = 143 : 55, $\chi^2$= 0.815 P = 0.367).

The segregation ratios of 3 : 1 observed in the $F_2$ population and the $\chi^2$ values strongly suggest that the dwarf type was controlled by a single recessive gene (Table 2). Previous studies have shown that dwarf type in T210 parent is inherited by a single recessive allele (Byth and Weber, 1969).

The Segregation for KTI protein and dwarf type in the $F_2$ generation was presented in Table 3. The segregation ratios of 9 : 3 : 3 : 1 (116 *Ti*_Df2_: 44 *Ti_df2df2*: 27 *titiDf2*_: 11 *tititidf2df2*) between *df2* gene and *ti* gene was observed ($\chi^2$= 3.53, P = 0.223). These results showed that *df2* gene was inherited independently with the *ti* gene in soybean.

Weiss (1970) reported *df2* gene was lined to *y11* gene. *Ti* gene was inherited independently with *Le* gene and $Cgy_1$ gene (Lee *et al.*, 2008; Sung *et al.*, 2010). This is the first evidence obtained from real data on independent inheritance between *df2* gene and *ti* gene.

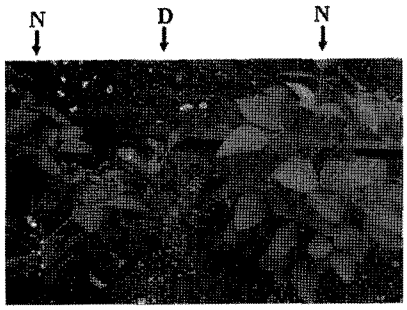


**Fig. 2.** Segregation of normal and dwarf type in $F_2$ plant generation obtained from the cross of Gaechuck#2" (*titiDf2Df2*) and T210 (*TiTidf2df2*).
N: normal type, D: dwarf type

**Table 2.** Observed and expected segregation of $F_2$ plants from selfed $F_1$ soybean plants from the cross "Gaechuck#2" (*titiDf2Df2*) and T210 (*TiTidf2df2*) for dwarf type.

| Parent-cross | Number of $F_2$ plants | | Expected | $\chi^2$ (3 : 1) | P |
|---|---|---|---|---|---|
| | Normal | Dwarf | | | |
| "Gaechuck#2" | all | | | | |
| T210 | | all | | | |
| F2 | 143 | 55 | 148.5 : 49.5 | 0.815 | 0.367 |

**Table 3.** Segregation of the presence or absence of Kunitz trypsin inhibitor (KTI) protein and dwarf type in $F_2$ generation from the crosses between "Gaechuck#2" (*titiDf2Df2*) and T210 (*TiTidf2df2*).

| Phenotype | | Number | | $\chi^2$ (9:3:3:1) | P |
|---|---|---|---|---|---|
| KTI protein | Dwarfness | Observed | Expected | | |
| Present | Normal | 116 | 111.375 | 3.53 | 0.223 |
| Present | Dwarf | 44 | 37.125 | | |
| Absent | Normal | 27 | 37.125 | | |
| Absent | Dwarf | 11 | 12.375 | | |

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