Accumulation of triple recessive alleles for three antinutritional proteins in soybean with black seed coat and green cotyledon

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Abstract The black seed coat of soybeans contain anthocyanins which promote health. However, mature soybean seeds contain antinutritional factors like lipoxygenase, lectin and Kunitz Trypsin Inhibitor (KTI) proteins. Furthermore, these seeds can be used only after the genetic elimination of these proteins. Therefore, the objective of this study was to develop novel soybean genotypes with black seed coat and triple recessive alleles ($l_{x1}l_{x2}l_{x3}, t_{i}t_{i}t_{i}$) for lipoxygenase, lectin, and KTI proteins. From a cross of parent1 ($l_{x1}l_{x2}l_{x3}/l_{x1}l_{x2}l_{x3}, t_{i}/t_{i}, L{e}/L{e}$) and parent2 ($l_{x1}l_{x2}l_{x3}/l_{x1}l_{x2}l_{x3}, T{i}/T{i}, l_{e}/l_{e}$), 132 $F_{2}$ seeds were obtained. A 3:1 segregation ratio was observed during $F_{2}$ seed generation for the inheritance of lectin and KTI proteins. Between a cross of the $L{e}$ and $T{i}$ genes, the observed independent inheritance ratio in the $F_{2}$ seed generation was 9:3:3:1 ($69 L_{e}T_{i}: 32 l_{e}l_{e}T_{i}: 22 L_{e}l_{e}l_{e}: 9 l_{e}l_{e}l_{e}$) ($\chi^{2} = 2.87$, $P = 0.5 − 0.1$). From nine $F_{2}$ seeds with triple recessive alleles ($l_{x1}l_{x1}l_{x2}l_{x2}l_{x3}l_{x3}, t_{i}t_{i}t_{i}$ genotype), one novel strain possessing black seed coat, and free of lipoxygenase, lectin and KTI proteins, was selected. The seed coat color of the new strain was black and the cotyledon color of the mature seed was green. The weight of 100 seeds belonging to the new strain was 35.4 g. This black soybean strain with $l_{x1}l_{x1}l_{x2}l_{x2}l_{x3}l_{x3}, t_{i}t_{i}t_{i}$ genotype is a novel strain free of lipoxygenase, lectin, and KTI proteins.

Keywords Lipoxygenase, Kunitz Trypsin Inhibitor, lectin, $l_{x1}l_{x1}l_{x2}l_{x2}l_{x3}l_{x3} t_{i}t_{i}t_{i}$ genotype

Introduction Soybean [Glycine max (L.) Merr.] protein is excellent nutritional factors and is widely used for human and animal feed in the world. In seed coat of black soybean, anthocyanins are especially abundant (Choung et al. 2001). Anthocyanins from soybean with black seed coat are known to have many pharmaceutical effect. Health-promoting effects such as reduction in the risk of coronary heart disease, regulation of adhesion molecules, protection from reperfusion, and potential antioxidant effects were reported (Burns et al. 2000; Kim et al. 2006). However, a few antinutritional factors such as lipoxygenase, lectin, and Kunitz trypsin inhibitor (KTI) proteins in raw mature soybean seed with black seed coat are present. These components reduce the nutritional value.

Lipoxygenases are a class of enzymes that catalyze the hydroperoxidation of polyunsaturated fatty acids such as the linoleic and linolenic acids. Lipoxygenase proteins constitute about 1 ~ 2% of the total protein. End-products from lipoxygenase activity are converted to many volatile compounds, which are responsible for the beany flavor in soybean products. Many researchers (Davies and Nielsen, 1986; Hildebrand and Hymowitz, 1981; Kitamura et al. 1983) have reported on the heredity and genetic elimination of lipoxygenase protein. Single dominant genes ($L_{x1}, L_{x2}$ and $L_{x3}$) control lipoxygenase protein and recessive alleles ($l_{x1}, l_{x2}, l_{x3}$) are responsible for absence of lipoxygenase protein in mature seed. Kobayashi et al. (1995) reported that soybean seeds with lipoxygenase free are better accepted due to production of very low levels of hexanal compounds. Macleod and Ames (1988) reported that extra costs need to inactivate lipoxygenase activity by heat at industrial level and the solubility and functionality of proteins was adversely affected. Breeding of soybean cultivar with lipoxygenase free through genetic elimination is the key to get rid of the beany flavour. So far, several cultivars with lipoxygenase free have been..
developed (Chung, 2009; Kim et al. 1997).

Kunitz (1945) isolated and crystallized Kunitz Trypsin Inhibitor (KTI) protein from soybean seeds for the first time. KTI protein is a small and non-glycosylated protein possessing 181 amino acid residues with 21.5 kDa. Trypsin is strongly inhibited by KTI protein and food intake is reduced by diminishing digestion and absorption. Four forms of \( Ti^{a}, Ti^{b}, Ti^{c}, \) and \( Ti^{d} \) have been reported at a single locus with a codominant multiple allelic series (Orf and Hymowitz, 1979; Hymowitz and Hadley, 1972; Sing et al. 1969). The fifth form as a recessive allele designated \( ti \) does not exhibit a soybean KTI protein (Orf and Hymowitz, 1979). Crude protein from \( titi \) genotype soybean had a 30 to 50% reduction in trypsin inhibitor activity compared with ‘Amsoy 71’ that has the \( TiTi \) genotype. The \( Ti \) gene has been located on chromosome 8 (molecular linkage group A2) of the USDA/Iowa State University soybean linkage map (Cregan et al. 1999; Hildebrand et al. 1980; Kiang, 1987).

Soybean agglutinin (lectin) protein is a major antinutritional element and can strongly endure degradation by proteases under in vitro and in vivo conditions. Soybean lectin protein is a glycoprotein that specifically binds galactose or N-acetylgalactosamine. Molecular weight of soybean lectin protein is 120 kDa (Pull et al. 1978). Soybean lectin protein contains four subunits that each have a molecular weight of 30 kDa. The concentration of lectins in soybean seed was ranged 1 ~ 2% on seed dry mass (George et al. 2008). By proper heating, the biological activity of soybean lectin protein can be reduced. However, considerable quantity is found after heating. The nutritional quality of the soybean protein was affected negativley by this residual soybean lectin and the digestion and absorption of nutrients was decreased (Schulze et al. 1995). By proper heating, the biological activity of soybean lectin protein can be reduced. However, considerable quantity is found after heating. The nutritional quality of the soybean protein was affected negatively by this residual soybean lectin and the digestion and absorption of nutrients was decreased (Schulze et al. 1995). Soybean seed lectin was controlled by a single gene designated \( Le \) (\( le \)) and \( lele \) genotype results in the lack of lectin in mature seed (Orf et al. 1978). Several researchers observed that \( Le \) and \( Ti \) loci were independently inherited (Lee et al. 2008; Moraes et al. 2006; Orf and Hymowitz, 1979). The soybean line with triple null recessive genotypes (\( ti/ti-le/\)
\( le-p34/p34 \)) was developed (Schmidt et al. 2015).

Presence of lipoxygenase, lectin, and KTI proteins in mature raw soybean seeds requires heating step to reduce the activity of these antinutritional components. But, excessive heat treatment may diminish amino acid availability. The genetic elimination of these factors could be an alternative to the heat treatment. New black soybean cultivars with free of lipoygenase, lectin, and KTI proteins improve the nutrition values and food processing properties of soybeans. This cultivar enhances the utilization of soybean in food as well as feed uses. The objective of this study was to improve new black soybean genotype with green cotyledon and triple recessive alleles (\( lx1lx1lx2lx2lx3lx3titilele \)) for lipoxygenase, lectin, and KTI proteins. This is the first report on black soybean line with green cotyledon and \( lx1lx1lx2lx2lx3lx3titilele \) genotype (free of lipoxygenase, lectin, and KTI proteins).

**Materials and Methods**

**Genetic population**

Four parents (“Gaechuck#1”, “Jimpum#2”, 12N1, and Le-16) were used to create genetic population. Genotype of “Gaechuck#1” is \( Lx1Lx1lx2lx2lx3lx3titiLeLe \) (lipoxygenase-2,3 and KTI proteins free and lectin protein present). “Jimpum#2” has \( bx1lx1lx2lx2lx3lx3TiTiLeLe \) genotype (lipoxygenase-1,2,3 protein free, KTI and lectin proteins present). 12N1 parent has \( bx1lx1lx2lx2lx3lx3TiTiLeLe \) genotype (lipoxygenase-1,2,3 protein free, KTI and lectin proteins present). Le-16 parent has \( Lx1Lx1lx2lx2lx3lx3TiTiLeLe \) genotype (abscence of lectin protein and presence of lipoygenase-1,2,3 and KTI proteins). Color of seed coat, presence or absence for lipoxygenase, lectin, and KTI proteins of four parents are presented in Table 1.

| Parents       | Seed coat color | Lipoxygenase | KTI     | Lectin |
|---------------|-----------------|--------------|---------|--------|
| Gaechuck#1    | Black           | 2,3 presence | Absence | Presence|
| Jimpum#2      | Yellow          | 1,2,3 absence| Presence | Presence|
| 12N1          | Black           | 1,2,3 absence| Presence | Presence|
| Le-16         | Yellow          | 1,2,3 presence| Presence | Absence|
were obtained. From F2 seeds, new parent2 with \( lx1lx1 \) \( lx2lx2lx3lx3 \) genotype (lipoyxygenase-1,2,3 and lectin proteins free, KTI protein present) was developed. From the cross of new parent1 and new parent2, F1 seeds were obtained and were planted in greenhouse. F2 seeds were harvested from F1 plants. The F2 seeds harvested from F1 hybrid plants were used to screen the seed with \( lx1lx1lx2lx2lx3lx3 \) genotype (lipoyxygenase, lectin, and KTI proteins free).

Identification of lipoyxygenase protein by SDS-PAGE

Total proteins from the parents, individual F2 seed, and random F4 seeds were obtained to identify the presence (‘+’) or absence (‘-’) of lipoyxygenase protein. A part of cotyledon from the parent, each F2 seed, and random F4 seed was removed and was incubated for 30 min in 1 ml Tris-HCl, pH 8.0 and 1.56% v/v β-mercaptoethanol. Through centrifugation, 50 μl of the supernatant was added to an equivalent amount of 5X sample buffer containing 1M Tris-HCl, pH 6.8, 50% v/v glycerol, 1.96% v/v β-mercaptoethanol, and 10% w/v sodium dodecyl sulfate (SDS). Sample obtained was boiled at 97°C for 5 min and sample was centrifuged. 2 μl of the supernatant was loaded on a 12% acrylamide SDS polyacrylamide gel electrophoresis medium gels in Owl Separation Systems Inc (Model: P9DS, Portsmouth, NH USA). After electrophoresis for 7 hrs at 120 V, gels were stained. For several hours, the gels were destained in destaining solution. Protein marker (Sigma MarkerTM, Product Code: M4038, St. Louis MO USA) was used to identify the presence or absence of lipoyxygenase protein (97 kDa).

Identification of lectin and KTI proteins by western blot analysis

Total proteins obtained from parental seeds, individual F2 seed, and random F4 seeds were separated by 10% or 12% SDS-PAGE, and transferred onto Immobilon-P membrane (PVDF, Millipore). After blocking for 2 hr in TBS buffer containing 0.1% Tween 20, 20 mM Tris (pH 7.5), 150 mM NaCl, and 5% nonfat dried milk (Carnation, Glendale, CA), the membrane were incubated with antibody of KTI and lectin protein for 1 hr. The blot was incubated with a horseradish peroxidase conjugated secondary antibody after washing in TBS buffer. Using enhanced chemiluminescence kit (Amersham, Bucking- hamshire, UK), the complex was visualized. Presence or absence of KTI and lectin protein was determined visually. The ratio of segregation for presence or absence of lectin and KTI proteins was determined by Chi-square analysis.

Improvement of soybean new strain with triple recessive and black seed coat

From the cross of new parent1 and new parent2, F2 seeds were obtained and were used to select the seed with \( lx1lx1lx2lx2lx3lx3 \) genotype (indicating absence of lipoyxygenase, lectin, and KTI proteins). The F2 seeds with triple null alleles (\( lx1lx1lx2lx2lx3lx3 \)) were planted to advance F2 plant generation. Each F2 plant with green cotyledon color and black seed coat color was harvested. F3 seeds with triple null alleles (\( lx1lx1lx2lx2lx3lx3 \)) were planted to advance F3 plant generation. Each F3 plant with a proper agronomical traits was harvested. Random F4 seeds obtained from F3 plants were used to confirm the absence of lipoyxygenase, lectin, and KTI proteins. Color of seed coat, hilum, and cotyledon was recorded on F4 seed. Seed weight (g/100 seeds) was recorded on the F4 plant generation. Scheme for improvement of \( lx1lx1lx2lx2lx3lx3, \) genotype (indicating absence of lipoyxygenase, lectin, and KTI proteins) is presented in Figure 1.

Results

From the cross of new parent1 (\( lx1lx2lx3lx1lx2lx3, ti/ti, Le/Le \)) and new parent2 (\( lx1lx1lx3lx1lx2lx3, Ti/Ti, le/le \)), 132 F2 seeds were obtained. Genotype of F1 seeds was
lx1lx1lx2lx2lx3lx3 and KTI and lectin proteins were observed. Lectin protein of 120 kDa and KTI protein of 21.5 kDa were segregated in the F2 seed generation. The data of segregation ratio for presence or absence at lectin and KTI proteins are presented in Table 2.

From 132 F2 seeds obtained, KTI protein was observed in 101 F2 seeds and was not observed in 31 F2 seeds. The segregation ratio of 3:1 was observed in the F2 seed generation (χ²=0.16, P=0.9 - 0.5). From 132 F2 seeds obtained, lectin was observed in 91 F2 seeds and was not observed in 41 F2 seeds. For inheritance of lectin protein, the 3:1 segregation ratio was observed in the F2 seed generation (χ²=2.59, P=0.5 - 0.1).

Between Le allele and Ti allele, segregation ratio of 9 : 3 : 3 : 1 (69 Le_Ti_; 32 lele_Ti_; 22 Le_titi; 9 leleti) was observed (χ²=2.87, P=0.5 - 0.1) in the F2 seed generation. Nine F2 seeds with triple recessive alleles (lx1lx1lx2lx2lx3lx3, titilele) were planted and one seed was not germinated. Each F2 plant with black seed coat color and green cotyledon color was harvested. Total four F2 plants were selected. Random F3 seeds of each F2 plant strain were planted to advance F3 plant generation. One F3 plant line possessing a proper agronomical traits among four strains was selected and was harvested. Random F4 seeds were used to identify the free for lipoxygenase, lectin, and KTI proteins (Fig. 2).

The absence for lipoxygenase, lectin, and KTI proteins was confirmed in protein extracted from random F4 seeds of new strain. However, lipoxygenase, lectin, and KTI proteins were observed in the seed of “Chungja#3” (Lx1Lx1Lx2Lx2Lx3Lx3, TiTiLeLe genotype) cultivar (Fig. 2). Color of seed coat, hilum, and cotyledon was recorded on F5 seed. Seed weight (g/100 seeds) was recorded on the F4 plant generation. F5 seeds harvested from F4 plant strain with triple null alleles (lx1lx1lx2lx2lx3lx3, titilele) are shown in Figure 3. Color of seed coat for new strain was black and color of cotyledon in mature seed was green. The 100-seed weight (g) of new strain was 35.4.

**Discussion**

Soybean seeds contain 40% protein, 20% oil, 30% carbohydrate, anthocyanin, saponin, and many other nutrients to human food and animal feed. By high quantity and quality of soybean protein, demand of soybean and soybean products has increased in recent years. However, a few antinutritional factors and allergenic proteins are exist in the raw mature soybean. Lipoxygenase protein, lectin protein, and Kunitz Trypsin Inhibitor (KTI) protein are major antinutrients affecting in reducing functional or nutritional value of unprocessed soybean. To denature the activity of these antinutritional components, heating step is necessary. However, excessive heat process may lower amino acid availability of soybean and soybean products. The genetic elimination of these antinutritional components could be

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**Table 2** Heredity pattern for the presence or absence of lectin and KTI proteins in the F2 seed generation

| Seed protein | Number of seed | χ² value (9:3:3:1) | P       |
|--------------|----------------|-------------------|---------|
| KTI Lectin   | Observed       | Expected          |         |
| + +          | 69             | 74.25             |         |
| + -          | 32             | 24.75             |         |
| - +          | 22             | 24.75             | 2.87    | 0.5 - 0.1 |
| - -          | 9              | 8.25              |         |

+: presence, -: absence

Fig. 2 Verification of the absence of lipoxygenase protein (Ⓐ), Kunitz Trypsin Inhibitor (KTI) protein (Ⓑ), and lectin protein (Ⓒ) in the current cultivar (“Chungja#3”) and new strain. C: “Chungja#3” (Lx1Lx1Lx2Lx2Lx3Lx3, TiTiLeLe genotype), S: new strain (lx1lx1lx2lx2lx3lx3, titilele genotype). +, -: presence or absence of lipoxygenase, lectin, and KTI proteins, respectively

Fig. 3 Physical appearance of F3 seeds possessing triple recessive alleles (lx1lx1lx2lx2lx3lx3, titilele) expressing black seed coat and green cotyledon, with the absence of lipoxygenase, lectin, and KTI proteins

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an alternative to the severe heat process. From the cross of new parent1 (lx1lx2lx3lx1lx2lx3, ti/ti, Le/Le) and new parent2 (lx1lx2lx3lx1lx2lx3, Ti/Ti, le/le), 132 F2 seeds were obtained to develop a new soybean line with black seed coat color, green cotyledon color, and triple recessive alleles for lipoxygenase, lectin, and KTI proteins. The ratios of segregation for presence and absence of lectin and KTI proteins are presented in Table 2. A 3:1 segregation ratio was observed ($\chi^2=0.16, P=0.9 - 0.5$) for the presence or absence of KTI protein in the F2 seed generation. Many researchers observed that the presence or absence of KTI protein is controlled by a single gene (Choi et al. 2016; Eun et al. 2012; Kim et al. 2006; Orf and Hymowitz, 1979). Also, a 3:1 segregation ratio was observed ($\chi^2=2.59, P=0.5 - 0.1$) for the presence or absence of lectin protein in the F2 seed generation. This result substantiate previous observations that lectin protein is controlled by a single gene (Choi et al. 2016; Orf and Hymowitz, 1979; Sung et al. 2013).

Between Le gene and Ti gene, segregation ratio of 9 : 3 : 3 : 1 (69 Le_Ti_ : 32 lele Ti_ : 22 Le_titi : 9 leletit) was observed ($\chi^2=2.87, P=0.5 - 0.1$) in the F2 seed generation. This result agreed with previous papers that both Ti and Le alleles were independently inherited (Choi et al. 2016; Lee et al. 2008; Moraes et al. 2006; Orf and Hymowitz, 1979). Independent inheritance of Ti and Le loci was observed in F2 population consisted with 24 plants (Moraes et al. 2006). Orf and Hymowitz (1979) reported that Le and Ti alleles were inherited independently by using F2 population with 96 plants. Lee et al. (2008) reported that Ti and Le alleles were independently inherited in 173 F2 seed generation. Also, Choi et al. (2016) observed that Le and Ti alleles were independently inherited in F2 seed generation consisted with 179 seeds.

Nine F2 seeds with triple recessive alleles (lx1lx1lx2lx2lx3lx3titilele genotype) from 132 F2 seeds were selected. One F2 plant line possessing a proper agronomical traits among four strains was obtained. Random F4 seeds were used to identify the presence or absence for lipoxygenase, lectin, and KTI proteins (Fig. 2). The absence for lipoxygenase, lectin, and KTI proteins was confirmed at the mature F4 seeds of new strain. However, in the seed of “Chungja#3” (lx1lx1lx2lx2lx3lx3 TiTILeLe genotype) cultivar, lipoxygenase, KTI, and lectin proteins were observed (Fig. 2). F3 seeds with triple recessive alleles (lx1lx1lx2lx2lx3lx3titilele) are shown in Figure 3. Color of seed coat for new strain was black and color of cotyledon was green in mature seed. The 100-seed weight (g) for new strain was 35.4. This is the first new black soybean strain with lx1lx1lx2lx2lx3lx3titilele genotype (absence of lipoxygenase, lectin, and KTI proteins). The strain improved newly in this study will be used to develop new soybean cultivar with black seed coat, green cotyledon, lipoxygenase protein free, KTI protein free, lectin protein free, and high quality.

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Reference

Burns J, Gardner PT, O’Neil J, Crawford S, Morecroft I, McPhail DB, et al. (2000) Relationship among antioxidant activity, vasodilatation capacity and phenolic contents of red wine. J. Agric. Food Chem. 48:220-230
Choi SW, Han SJ, Sung MK, Chung JI (2016) Breeding of black soybean line with ti and le allele. Plant Breed. Biotech. 4(2):170-175
Choung MG, Baek IY, Kang ST, Han WY, Shin DC, Moon HP, et al. (2001) Isolation and determination of anachocyanins in seed coats of black soybean (Glycine max (L.) Merr.). J. Agric. Food Chem. 49:5848-5851
Chung JI (2009) A new cultivar “Gaeheuck#1”: black soybean cultivar with lipoygenase2,3-free, Kunitz trypsin inhibitor-free and green cotyledon. Korean J. Breed. Sci. 41(4):603-606
Cregan PB, Jarvik T, Bush AL, Shoemaker RC, Lark KG, Kahler AL, et al. (1999) An integrated genetic linkage map of the soybean genome. Crop Sci. 39:1464-1490
Davies CS, Nielsen SS (1986) Genetic analysis of a null-allele for lipoygenase -2 in soybean. Crop Sci. 26:460-462
Eun HH, Sung MK, Baek WJ, Shin SI, Kim MC, Chung JI (2012) Inheritance of Kunitz trypsin inhibitor and P34 protein in soybean seed. Korean J. Crop Sci. 57(1):78-82
George M, Bhide S, Thengane R, Hosseini G, Manjaya J (2008) Identification of low lectin mutants in soybean. Plant Breed. Biotech. 127:150-153
Hidebrand DF, Orf JH, Hymowitz T (1980) Inheritance of an acid phosphatase and its linkage with the Kunitz trypsin inhibitor in seed protein of soybeans. Crop Sci. 20:83-85
Hildebrand DF, Hymowitz T (1981) Soybeans lacking lipoxygenase. J. Am. Oil Chem. Soc. 58:583-586
Hymowitz T, Hadley HH (1972) Inheritance of a trypsin inhibitor variant in seed protein of soybeans. Crop Sci. 12:197-198
Kiang YT (1987) Mapping three protein loci on a soybean chromosome. Crop Sci. 27:44-46
Kitamura K, Davies CS, Kaizuma N, Nielsen NC (1983) Genetic
analysis of a null-allele for lipoxygenase-3 in soybean seeds. Crop sci. 58:583-586
Kim HJ, Tsoy I, Park JM (2006) Anthocyanins from soybean seed coat inhibit the expression of TNF-alpha-induced genes associated with ischemia / reperfusion in endothelial cell by NF-kappaB-dependent pathway and reduce rat myocardial damages incurred by ischemia and reperfusion in vivo. FEBS Lett. 580:1391-1397
Kim SD, Kim YH, Park KY, Yun HT, Lee SH, Lee YH, et al. (1997) A new beany tasteless soybean variety “Jinpumkong 2” with good quality. Korean J. Breed. 29(4):502
Kobayashi AT, Hirata N, Kubota K, Kitamura (1995) Aroma constituents of soybean milk lacking lipoxygenase isozymes. J Agri Food Chem 43:2449-2452
Kunitz M (1945) Crystallization of a soybean trypsin inhibitor from soybean. Science 101:668-669
Lee KJ, Park MS, Sung MK, Kim MS, Chung JI (2008) Inheritance between Le gene and Ti gene in soybean (Glycine max L.). Korean J. Breed Sci. 40(2):97-100
MacLeod G, Ames J (1988) Soya flavor and its improvement. CRC Crit Rev Food Sci Nutr. 27:219-400
Moraes RMA, Soares TCB, Colombo LR, Salla MFS, Barros JGA, Piovesan ND, et al. (2006) Assisted selection by specific DNA markers for genetic elimination of the kunitz trypsin inhibitor and lectin in soybean seeds Euphytica 149:221-226
Orf JH, Hymowitz T, Pull SP, Pueppke SG (1978) Inheritance of a soybean seed lectin. Crop Sci. 18:899-900
Orf JH, Hymowitz T (1979) Inheritance of the absence of the Kunitz trypsin inhibitor in seed protein of soybeans. Crop Sci. 19:107-109
Orf JH, Hymowitz T (1979) Soybean linkage test between Ti and Le seed proteins. Soybean Genetics Newsletter Vol. 6:32
Pull SP, Pueppke SG, Hymowitz H, Orf JH (1978) Soybean lines lacking the 120,000 daltons seed lectin. Science 200:1277-1279
Schulze H, Saini HS, Huisman J, Hessing M, Berg W, Verstegen MWA (1995) Increased nitrogen secretion by inclusion of soya lectin in the diets of pigs. J. of Science Food and Agriculture 69:501-510
Schmidt MA, Hymowitz T, Herman EM (2015) Breeding and characterization of soybean triple null; a stack of recessive alleles of Kunitz Trypsin Inhibitor, Soybean Agglutinin, and P34 allergen nulls. Plant Breeding. 134:310-315
Singh L, Wilson CM, Hadley HH (1969) Genetic differences in soybean trypsin inhibitors separated by disc electrophoresis. Crop Sci. 9:489-491
Sung MK, Kim MH, Seo HJ, Chung JI (2013) Inheritance of dlm and ti genes in soybean. Plant Breed. Biotech. 1(1):9-13