SURVEY OF GMO SOYBEANS IN TIEN GIANG PROVINCE AND COMPARISON OF PROTEIN AND LIPID CONTENT WITH NON-GMO SOYBEANS

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ABSTRACT

The objective of this study is to determine the presence of genetically modified (GMO) soybean varieties in markets within Tien Giang province, and to compare the total protein and lipid content of these GMO soybeans with non-GMO soybean varieties. Twenty-one soybean varieties collected from various markets in Tien Giang province were subjected to genetic analysis. PCR was employed using specific primers targeting the 35S promoter and nos terminator sequences to detect the presence of genetically modified traits. Results showed that 52.38% of the 21 soybean varieties tested were genetically modified organisms (GMOs). The mean growth duration of these soybean cultivars was relatively short, approximately 61 days. A strong positive correlation was observed between plant height and node number. The soybean variety with the highest protein content is CG1 (0.67 %). The variety with the lowest lipid content is TP3 (8.71%).

1. INTRODUCTION

With a growing population and a changing climate, ensuring food security is a significant challenge. Genetically modified crops, with their high adaptability and stable yields, offer a promising new direction in agriculture. Soybeans, with their abundant protein content and adaptability, are a prime example. Research and development in genetically modified crops not only increase yields but also improve nutritional quality, meeting the growing demands of the human population. Vietnam's membership in the World Trade Organization since 2006 has necessitated the implementation of strict regulations governing genetically modified organisms. In line with Prime Minister's Decision No. 212/2005/QD-TTg [1], all GMO products, whether imported, exported, or sold domestically, must bear a clear label indicating the presence of genetically modified ingredients with the statement: "Product contains genetically modified organisms". This regulatory framework is designed to ensure transparency and protect consumer rights.

A survey conducted by Quatest 3 in 2010 on 323 randomly had selected food samples (including corn, soybeans, potatoes, rice, tomatoes, and peas) from 17 local markets and supermarkets in Ho Chi Minh City revealed that 111 samples (34.36%) were genetically modified. This suggests that GMOs have become a common component of the Vietnamese food supply. With the increasing influx of imported agricultural products into the market, accurately determining their origin and composition, especially the presence of GMOs, has become crucial. Therefore, implementing molecular biological testing for these products is essential to ensure food safety and transparency for consumers.

In Vietnam, there have been numerous studies on genetically modified crops, including the development of pest-resistant and drought-tolerant varieties. However, research and evaluation of genetically modified crops remain limited and uncommon in provinces nationwide,

especially in Tien Giang. Based on this reality, we conducted a study titled "A survey on the presence of Genetically Modified (GMO) soybeans in several markets in Tien Giang province and a comparison of protein and lipid content between these and non-GMO soybeans" by using molecular biology techniques, aiming to detect and analyze plant products of genetically modified origin.

2. MATERIALS AND METHODS

2.1 Research materials

Soybean samples (n=21) were collected from local markets in Tien Giang province, including My Tho (MT), Cai Lay (CL), Cai Be (CB), Cho Gao (CG), Tan Phuoc (TP), Tan Ly Dong (TLĐ), Tan Ly Tay (TLT), and Tan Hiep (TH). The collected soybean samples are illustrated in Figure 1.

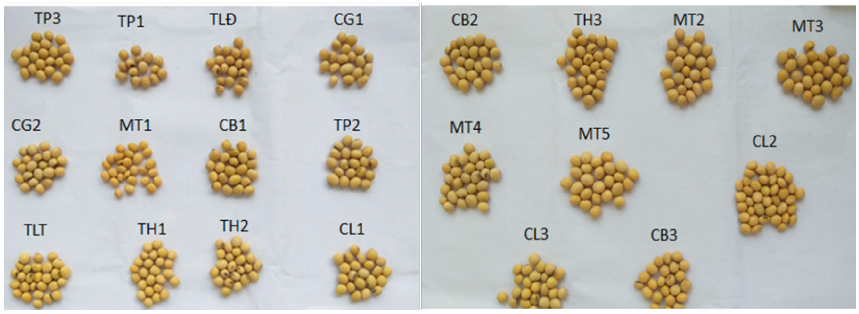

Figure 1. Soybean samples collected from various markets in Tien Giang Province

Specific primers for the detection of the 35S promoter and Terminator nos sequence and were obtained [2] and synthesized by Phu Sa Company.

The 35S promoter sequence:

Reverse primer: 5’ GAT AGT GGG ATT GTG CGT CA 3’.

Forward primer: 5’ GCT CCT ACA AAT GCC ATCA 3’.

Terminator nos sequence:

Reverse primer: 5’ GAC ACC GCG CGC AGT AAT TTA TCC 3’.

Forward primer: 5’ GCA TGA CGT TAT TTA TGA GAT GGG 3’.

PCR eppedorf Mastercycler nexus gradient X2-Germany, Agarose *gel* electrophoresis Chemidoc XRS-USA, Master mix 2X – Phu Sa Company, Safe view DNA –IBM.

2.2. Molecular biology techniques

2.2.1. DNA extraction technique

Soybean seeds were sown and grown until seedlings reached a height of 20 cm. Leaves were harvested, rinsed with sterile distilled water, and sterilized with 70% ethanol. Total genomic DNA was extracted from leaf samples by using a modified CTAB protocol [3].

2.2.2. Genetically modified soybean detection method based on specific primer pairs designed by PCR technique

Genomic DNA had isolated from leaf tissues was amplified via PCR by using 35S promoter and nos terminator primers to detect genetically modified soybean genotypes, adhering to the protocol outlined [4]. The PCR reaction mixture comprised: 1X (2X Master mix), 1 µM (forward and reverse primers), and 2 µL DNA template.

Thermal cycling is performed to amplify in PCR with the 35S primers as shown in Figure 2 Thermal cycling is performed to amplify in PCR with the Nos terminator primers as shown in Figure 3.

In this study, we followed the protocol of Tran Thi My Hien [5] to detect the 35S promoter and Terminator nos. A positive result was indicated by the presence of a 195 bp (35S promoter) band and 118bp band (Terminator nos) on the agarose gel.

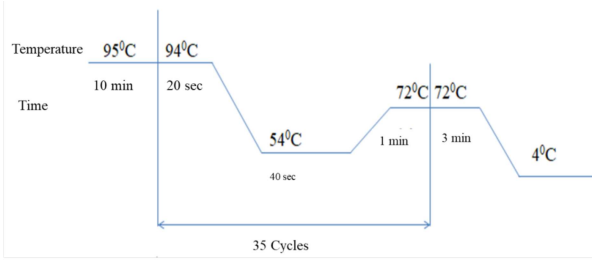

Figure 2. PCR reaction scheme with 35S promoter primer

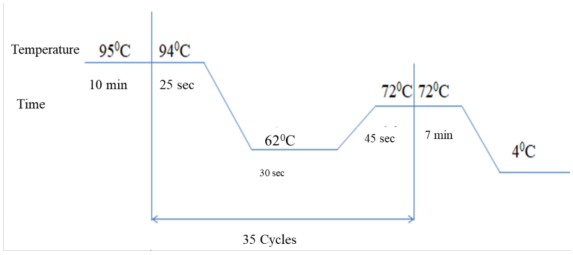

Figure 3. PCR reaction scheme with Nos terminator promoter primers

2.2.3. Experimental design for the study of GMO and non-GMO soybeans focusing on agronomic traits, protein, and lipid content

Experimental design: After identifying GMO and non-GMO soybean varieties, GMO varieties (6 samples) and non-GMO varieties (3 samples) were randomly selected. The selected soybean seeds were sown in 20 cm tall pots, with 3 seeds per pot for each variety. The treatments were numbered from 1 to 6 for GMO varieties and from 7 to 10 for non-GMO control varieties. This experiment consisted of 10 treatments with 3 replicates for each treatment.

Monitoring parameters: Data on traits and phenotypes were recorded for each individual. These parameters were referenced from the guidelines of the Asian Vegetable Research and Development Center (AVRDC), 1979. The number of plants sampled and units of measurement were adjusted to fit our specific study. Morphological traits such as seedling stem color, flower color, leaf color, fruit peel color, and seed color were recorded. Quantitative traits recorded included: the number of fruits and the number of seeds.

2.2.4. Analytical methods for soybean protein and lipid quantification

This study had employed the Kjeldahl method [6] for protein determination and Soxhlet [7] extraction for lipid analysis in soybean samples to compare the nutritional value of genetically modified and non-GMO varieties.

2.2.5. Statistical analysis methods

Agronomic data would be analyzed using Microsoft Excel for basic statistical analysis. Means would be compared using ANOVA followed by Duncan's multiple range test, performed using SPSS 16.0 to compare mean values and identify significant differences among treatments.

3. RESULTS AND DISCUSSION

3.1. PCR analysis of soybean samples using 35S promoter and nos terminator primers

In plant genetic engineering, promoters are extensively used to control the expression of transgenes. The 35S promoter and NOS terminator are two widely employed promoter elements, as demonstrated [8]. The presence of GMOs can be detected through PCR amplification using primers specific to the

35S promoter and NOS terminator, yielding distinct bands at 195 bp and 118 bp, respectively provide conclusive evidence of GMOs when visualized on agarose gels.

*PCR products analysis with the 35S promoter

To confirm the presence of the target gene in the transgenic soybean plants, genomic DNA was extracted from leaf tissues and subjected to PCR amplification using primers specific for the 35S promoter region. PCR products from soybean DNA samples, amplified with 35S promoter primers, were visualized on a 1.5% agarose gel by safe view.

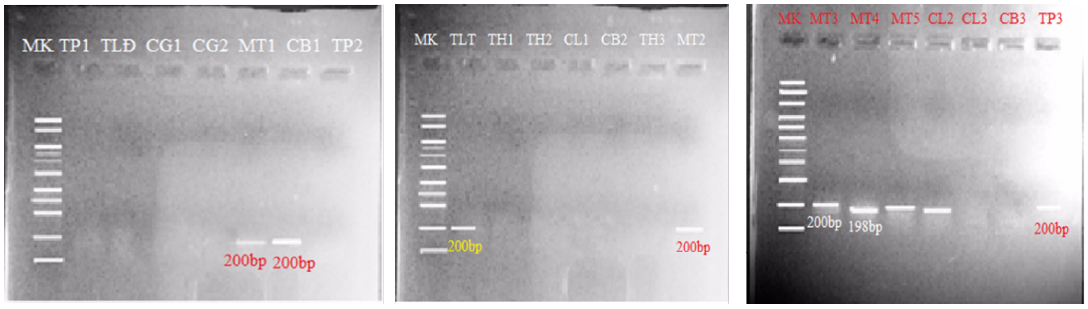

Figure 4. Analysis of PCR products amplified with 35S promoter primers by 1.5% agarose gel electrophoresis

Note: *The abbreviations used in this study are as follows: MK for marker, TP1 for Tan Phuoc 1, TLĐ for Tan Ly Dong, CG1 for Cho Gao 1, CG2 for Cho Gao 2, MT1 for My Tho 1, CB1 for Cai Be 1, CB2 - Cai Be 2; TH3 - Tan Hiep 3; MT2 - My Tho 2; MT3 - My Tho 3; MT4 - My Tho 4; MT5 - My Tho 5; CL2 - Cai Lay 2; CL3 - Cai Lay 3; CB3 - Cai Be 3; TP3 - Tan Phuoc 3.*

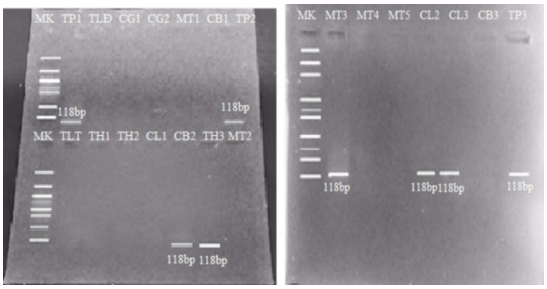

Figure 5. Analysis of PCR products amplified with terminator nos promoter primers by 1.5% agarose gel electrophoresis

Note: *The abbreviations used in this study are as follows: MK for marker, TP1 for Tan Phuoc 1, TLĐ for Tan Ly Dong, CG1 for Cho Gao 1, CG2 for Cho Gao 2, MT1 for My Tho 1, CB1 for Cai Be 1, CB2 - Cai Be 2; TH3 - Tan Hiep 3; MT2 - My Tho 2; MT3 - My Tho 3; MT4 - My Tho 4; MT5 - My Tho 5; CL2 - Cai Lay 2; CL3 - Cai Lay 3; CB3 - Cai Be 3; TP3 - Tan Phuoc 3.*

Analysis of PCR products from soybean samples by agarose gel electrophoresis indicated that genotypes with potential transgene insertion displayed bands corresponding to either a 200bp or 198bp fragment. A total of eight genotypes, namely MT1, CB1, TLT, MT2, MT3, MT4, MT5, and CL2, were positive for the transgene. Conversely, the absence of bands in genotypes such as TP1, TLĐ, CG1, CG2, TP2, TH1, TH2, TH3, CL1, and CB2 suggested the lack of transgene integration.

*PCR products analysis with the terminator nos promoter

Genomic DNA was isolated from leaf tissues of soybean plants to verify the presence of the target gene. PCR amplification was performed using primers specific to the terminator nos promoter region. Subsequently, the PCR products obtained from soybean DNA samples were visualized on a 1.5% agarose gel.

Agarose gel electrophoresis of PCR products using terminator nos primers revealed the presence of a 118 bp DNA band, characteristic of transgenic soybean genotypes. This result indicated that 8 genotypes, namely TP1, TP2, CB2, TH3, MT3, CL2, CL3, and TP3, carried the transgene. Conversely, the remaining

genotypes did not exhibit a band at this position, suggesting the absence of the transgene.

Table 1. Comparison of gel electrophoresis results using two different primer sets

Genotype	Promoter 35S	Terminator nos	Result
TP1	-	+	Positive
TLĐ	-	-	Negative
CG1	-	-	Negative
CG2	-	-	Negative
MT1	+	-	Positive
CB1	+	-	Positive
TP2	-	-	Negative
TLT	+	+	Positive
TH1	-	-	Negative
TH2	-	-	Negative
CL1	-	-	Negative
CB2	-	-	Negative
TH3	-	-	Negative
MT2	+	+	Positive
MT3	+	+	Positive
MT4	+	-	Positive
MT5	+	-	Positive
CL2	+	+	Positive
CL3	-	+	Positive
CB3	-	-	Negative
TP3	-	+	Positive

A summary of the results is presented in the table 1. Genotypes were categorized as positive (+) or negative (-) based on PCR amplification using both primer pairs. A genotype was considered positive if a band was observed with at least one of the primer pairs.

PCR amplification using both the 35S promoter and terminator nos primer pairs was carried out to detect the presence of the transgene. Eleven out of the total genotypes tested positive, as indicated by the presence of a PCR product. The positive genotypes were TP1, MT1, CB1, MT2, MT3, MT4, MT5, TLT, CL2, CL3, and TP3. The remaining ten genotypes, including CB3, TH1, TH2, CL1, CB2, TH3, TLĐ, CG1, CG2, and TP2, were negative.

3.2. Morphological trait analysis

Analysis results of agronomic traits such as yield, plant height, and number of seeds. To investigate phenotypic differences between genetically modified and non-GMO genotypes, a random sample of 6 GMO genotypes (TP3, CB1, TLT, MT2, MT3, CL2) and 4 non-GMO genotypes (CG1, TH2, CL1, CB3) were selected for further analysis. The results of the phenotypic characterization are summarized in Table 2.

Previous research by Luong Tien Si [9] reported a linkage between flower color and hypocotyl color in soybeans, with purple-hypocotyl plants typically producing purple flowers and green-hypocotyl plants producing white flowers. However, our experimental results showed that 100% of the soybean genotypes examined produced purple flowers. This can be explained by the simultaneous occurrence of both purple and white flowers within the same genotype, as observed in CB1, MT3, and CL1, with a white flower ratio of approximately 30%.

This result aligns with the findings of Takahashi and Fukuyama [10] suggesting a genetic linkage between hypocotyl color and flower color. The W1 allele is believed to control both purple flower color and purple hypocotyl color, with purple hypocotyl being dominant over green hypocotyl, which is controlled by the w1 allele.

Immature soybean pods typically exhibit a green color. However, upon maturation, the pod color transitions to specific hues characteristic of each cultivar, primarily yellow-brown and brown. Our research on 10 soybean genotypes revealed that 90% of the genotypes possessed yellow-brown pods, while the remaining 10% exhibited brown pods.

The seed coat plays a crucial role in protecting the embryo during storage. The presence of anthocyanin pigments within the seed coat determines the diverse range of colors, including yellow, brown, green, and black, as reported Truong Trong Ngon [11]. Our study revealed that in most soybean cultivars, the predominant seed coat color was yellow, accounting for approximately 90%. However, a minority of cultivars, such as CL2, exhibited a darker yellow seed coat, representing about 10% of the total.

Table 2. Morphological trait analysis results

No.	Origin	Leaf color	Flower color	Fruit color	Seed color
1	TP3	Green	Purple	Yellow-brown	Yellow
2	CB1	Green	White, purple	Yellow-brown	Yellow
3	TLT	Green	Purple	Yellow-brown	Yellow
4	MT2	Green	Purple	Yellow-brown	Yellow
5	MT3	Green	White, purple	Yellow-brown	Yellow
6	CL2	Green	Purple	Brown	Dark yellow
7	CG1	Green	Purple	Yellow-brown	Yellow
8	TH2	Green	Purple	Yellow-brown	Yellow
9	CL1	Green	White, purple	Yellow-brown	Yellow
10	CB3	Green	Purple	Yellow-brown	Yellow

Analysis of variance (ANOVA) and Duncan's multiple range test at the 1% significance level revealed significant differences among genotypes for several quantitative traits. The number of pods per plant varied from 3 to 28, although no significant differences were found among genotypes. However, the number of pods influenced the number of seeds per plant. Each pod contained 1 to 4 seeds, with most pods containing 1 or 2 seeds.

Figure 6. Phenotypic variation in flower color among 10 genotypes. (A) Purple flowers, (B) White flowers

Figure 7. Phenotypic variation in pod color and seed coat among 10 genotypes. (A) Green immature pods, (B) Mature pods with yellow-brown and (C) brown hues, (D) Dark yellow seed coat, (E) Light yellow seed coat.

The number of seeds per plant, a determinant of yield, ranged from 6 to 64 seeds. Genotype CB3 had the lowest mean number of seeds (6.44 seeds), significantly different from the other genotypes. Conversely, genotype CL2 had the highest mean number of seeds (31.33 seeds) and also the highest number of pods. An ideal bean genotype should have both a high number of pods and seeds per pod. Correlation analysis revealed a strong positive correlation between the number of pods and the number of seeds ($r = 0.901$ at the 1% significance level). Therefore, to select for superior genotypes with high pod and seed numbers and low rates of empty pods and single-seeded pods, attention should be paid to cultivation practices.

Table 3. Comparison of pod and seed number among 10 soybean genotypes

Genotype	Mean number of seeds (range)	Mean number of pods (range)
TP3	1.1398 (14.22)^{ab}	0.827874 (6.89)^{ab}
CB1	1.0035 (10.89)^{bc}	0.789205 (6.78)^{ab}
TLT	0.9317 (9.33)^{bc}	0.490404 (3.33)^{c}
MT2	1.218 (18.33)^{ab}	0.868870 (8)^{ab}
MT3	1.2903 (20.33)^{a}	0.90081 (8)^{a}
CL2	1.3675 (31.33)^{a}	0.974298 (13.56)^{a}
CG1	1.3444 (23.44)^{a}	0.916415 (8.78)^{a}
TH2	1.1096 (13.67)^{ab}	0.846417 (7.33)^{ab}
CL1	0.9707 (10)^{bc}	0.603485 (4.11)^{bc}
CB3	0.7631 (6.44)^{c}	0.473952 (3.22)^{c}
Mean	1.11	0.77
F	8.011**	0.039**
CV(%)	18.9	25.65

Duncan's multiple range test following analysis of variance indicated that means with the same letter do not differ significantly at the 1% level. This implies that genotypes sharing the same letter are not significantly different in terms of seed and pod number.

3.3. Analysis of protein and lipid content

Table 4. Comparison of protein and lipid content among 10 experimental genotypes

Genotype	Mean protein content (%)	Mean lipid content (%)
TP3	0.51^{abc}	8.71^{d}
CB1	0.39^{bc}	18.06^{ab}
TLT	0.55^{ab}	12.59^{cd}
MT2	0.38^{bc}	18.24^{ab}
MT3	0.36^{bc}	18.47^{ab}
CL2	0.33^{bc}	16.99^{bc}
CG1	0.67^{a}	18.24^{ab}
TH2	0.33^{bc}	13.52^{bcd}
CL1	0.44^{bc}	13.56^{bcd}
CB3	0.31^{c}	19.06^{a}
F	2.87*	10.57**
CV (%)	26.89	9.5

Duncan's multiple range test following analysis of variance indicated that means with the same letter do not differ significantly at the 1% level. This implies that genotypes sharing the same letter are not significantly different in terms of seed and pod number.

Seed quality of soybean was evaluated not only based on morphological characteristics but also on biochemical composition, specifically protein and lipid content. The results showed that the average protein content of the genotypes ranged from 0.31% (CB3) to 0.67% (CG1). The differences in protein content among genotypes were statistically significant at the 5% level. Genotype CG1 had the highest protein content and was significantly different from the other genotypes, indicating its potential as a parental line for improving protein content. Lipid content also varied significantly among genotypes, ranging from 8.71% (TP3) to 19.06% (CB3), and this difference was statistically significant at the 1% level. However, the average lipid content of the genotypes in this study was lower than the results reported [12], with an average value below 20%.

4. CONCLUSION AND SUGGESTIONS

PCR with specific primers targeting the 35S promoter and nos terminator was employed to detect the presence of transgenic events in soybean samples collected from markets in Tien Giang province. The results revealed a high prevalence of genetically modified organisms in the samples, accounting for 52.38% of the total. The areas with the highest proportion of GMO-positive samples were My Tho, Tan Phuoc, and Cai Lay. In contrast, samples collected from Tan Hiep, Cai Be, and Cho Gao were predominantly GMO-free.

Interestingly, despite the high prevalence of GMOs, some traditional soybean varieties such as

Cai Lay 2 and Cho Gao showed high potential in terms of agronomic traits, particularly Cho Gao 1 with the highest protein content (0.67373 mg%). This suggests that the conservation and development of traditional soybean varieties remain crucial, alongside the management and utilization of genetically modified varieties.

To gain a more comprehensive understanding of the status of GMO soybean usage in Vietnam, it is necessary to expand the research to other provinces. This will help build a comprehensive database, thereby supporting the development of more effective policies for the management and utilization of GMO soybeans.

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KHẢO SÁT SỰ CÓ MẶT CỦA ĐẬU NÀNH GMO TRONG TỈNH TIỀN GIANG VÀ SO SÁNH HÀM LƯỢNG ĐẠM, LIPID VỚI ĐẬU NÀNH KHÔNG CHỨA GMO

TÓM TẮT

Mục tiêu của nghiên cứu là xác định sự có mặt của đậu nành biến đổi gen trong các chợ của tỉnh Tiền Giang và so sánh hàm lượng đạm tổng số, hàm lượng lipid tự do của đậu nành chứa GMO và đậu nành không chứa GMO. Vật liệu thí nghiệm gồm 21 giống thu thập tại các chợ trong tỉnh Tiền Giang. Công tác kiểm nghiệm sinh học phân tử trong phân tích các sản phẩm có nguồn gốc biến đổi gen là cần thiết nhằm giúp người tiêu dùng biết rõ nguồn gốc sản phẩm để đưa ra những quyết định sử dụng hợp lý hơn. Bằng việc sử dụng phương pháp PCR với các cặp mồi đặc hiệu để phát hiện trình tự Promoter 35S và Terminator nos đã cho ra kết quả là có đến 52,38% đậu nành GMO. Thời gian sinh trưởng trung bình của các giống tương đối ngắn (61 ngày). Chiều cao cây và số đốt có mối tương quan chặc chẽ với nhau. Giống có hàm lượng đạm cao nhất là CG1 (0,674 mg%). Giống có hàm lượng lipid thấp nhất là TP3 (8,713%).

Từ khóa: *Biến đổi gen, đạm đậu nành, GMO, lipid*