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# Molecular Marker Assisted Screening of soyabean germplasm for quality characteristics

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**ABSTRACT**

Soybean (*Glycine max*) is a one of the most valued crop in the world and an emerging crop in Nepal, with increasing cultivation in recent years in Nepal. The crop has gained popularity due to its high protein content and potential as a substitute for traditional protein sources, such as meat and dairy. Soybean is an important source of protein and oil and is widely cultivated for both food and industrial purposes. Marker-assisted screening (MAS) utilizing simple sequence repeat (SSR) markers has been used to pinpoint the genes that regulate the protein and oil content in soybeans. In this work, soybean germplasm were screened for the presence of particular markers associated with the genes regulating protein and oil using Five SSR markers (Satt556,Satt006,Satt212,Satt144 and Satt449) across eighty eight genotypes found in Nepal.The outcomes of this study showed promising results with presence of protein and oil genes on Nepalese genotypes indicating that SSR markers may be successfully utilized to pinpoint soybean genotypes with enhanced protein and oil content having potential increasing the quality and nutrition value.

**Keywords:** SSR markers, DNA Extraction, Polymerase Chain Reaction(PCR)

**सारांश**

भटमास नेपालमा अपेक्षाकृत नयाँ बाली हो, हालका वर्षहरूमा बढ्दो खेतीसँगै मासु र दुग्ध जस्ता परम्परागत प्रोटिन स्रोतहरूको विकल्पको कारण यो बालीले लोकप्रियता प्राप्त गरेको छ । भटमास प्रोटिन र तेलको महत्वपूर्ण स्रोत हो र खाद्य र औद्योगिक उद्देश्यका लागि व्यापक रूपमा खेती गरिन्छ । उत्पादन र गुणस्तर बढाउने प्रयासमा भटमासमा प्रोटिन र तेलको मात्रालाई विनियमित गर्ने जीनहरू पत्ता लगाउन मार्कर-असिस्टेड स्क्रिनिङ (MAS) प्रयोग गरिएको छ । यस कार्यमा SSR मार्करहरू प्रयोग गरेर प्रोटीन र तेल सामग्री नियमन गर्ने जीनसँग सम्बन्धित विशेष मार्करहरूको उपस्थितिको लागि संग्रहित भटमास जर्मप्लाज्महरूको जाँच गरियो । सुधारिएको पोषण मूल्यको साथ भटमास प्रजातिहरू सिर्जना गर्न, प्रजनन् कार्यक्रमहरूले यस जानकारीलाई मार्गदर्शनको रूपमा प्रयोग गर्न सक्दछ । भटमास प्रजनन्मा SSR मार्करको प्रयोगले यस प्रक्रियाको प्रभावकारितालाई उल्लेखनीय रूपमा बढाउने र राष्ट्रिय बजारको आवश्यकताहरू पूरा गर्न उच्च गुणस्तरको भटमास प्रजातिहरू उत्पादन गर्ने क्षमता राख्दछ ।

## INTRODUCTION

Soybean (*Glycine max* (L.) Merr) is a highly valuable crop that is widely cultivated worldwide for its high protein and oil content (Lam et al 2010). The crop has gained increasing popularity due to its potential as a substitute for traditional protein sources such as meat and dairy, and its versatility in different industries (Berger et al 2010). Soybean is an excellent source of essential amino acids, vitamins, and minerals, making them an important component of a balanced diet (Wang et al 2019). The cultivation of soybean is highly adaptable, making it suitable for cultivation in different regions worldwide (Sallam et al 2019). Soybean production has significantly increased in recent years, driven by the growing demand for plant-based protein (FAO 2019). In addition, the genetic improvement of soybean through breeding programs has been crucial in enhancing their yield, nutritional quality, and resistance to pests and diseases (Stupar et al 2016).

In Nepal, soybean cultivation has been increasing in recent years due to its high protein content and potential as a substitute for traditional protein sources, such as meat and dairy. Soybean was first introduced in Nepal in the early 1800's and its cultivation was done across the hilly and terai region with its local names and dishes being eaten commonly (Willam S and Akiko A 2010). However, in recent years, soybean has gained popularity among farmers due to its high yield potential, adaptability to different agro-climatic conditions, and market demand for its products. Currently, soybean is grown in various parts of Nepal, including the Terai region and the mid-hills. The Terai region, which is situated in the southern plains of Nepal, has favorable climatic and soil conditions for soybean cultivation. According to a study by Devkota et al (2019), soybean is mainly grown in the eastern Terai region of Nepal, particularly in Morang, Jhapa, Sunsari, and Saptari districts. The study also reported that the average yield of soybean in Nepal ranged from 1.2 to 2.2 t/ha, which is lower than the global average yield of 2.9 t/ha (FAOSTAT 2021). Despite the increasing popularity of soybean cultivation in Nepal, there are still challenges that need to be addressed to improve its production and yield. These challenges include the lack of improved varieties, limited access to quality seeds, inadequate pest and disease management practices, and low adoption of modern technologies (Devkota et al 2019).

Marker-assisted selection (MAS) has been widely used in soybean breeding programs to identify and select for desirable traits, such as improved nutritional quality and yield (Liu et al 2018). The use of molecular markers, such as simple sequence repeat (SSR) markers, has been particularly useful in identifying genes responsible for regulating the protein and oil content of soybean (Kwon et al 2017). Simple sequence repeat (SSR) markers have been utilized in marker-assisted screening (MAS) to identify soybean genotypes with enhanced protein and oil content. Breeders have reliably chosen desired features in segregating plant populations using the molecular marker strategy,compared to traditional phenotypic selection in the field. The earlier method (MAS) could save both time and money.

The purpose of this study was to identify good protein and oil linked genes using molecular markers (SSR markers) in an aim of effectively identify soybean genotypes having enhanced nutritional value with respect to Protein and oil .This study also act as a foundation for further research on varietial improvement using molecular techniques in Nepal. The use of MAS in soybean breeding has the potential to increase the effectiveness of this procedure and produce high-quality soybean varieties to satisfy the needs of the national market.

## MATERIALS AND METHODS

### Sample Collection

88 germplasm seeds collected form different sources were germinated and maintained on Naional Biotechnology Research Centre (NBRC), Nepal Agriculture Research Centre (NARC), Khumaltar, Lalitpur. ( Latitude: 27.65183 N , Longitude: 85.32527 E) (**Table** 1).

**Table 1. List of genotype accessions with Lab code used in this study**

| Lab code | Genotype Name | Lab code | Genotype Name | Lab code | Genotype Name | Lab code | Genotype Name |
|---|---|---|---|---|---|---|---|
| S1 | NGRC02687 | S23 | NGRC02703 | S45 | 2003KS-TB1xKB-5.4 | S67 | NGRC02672 |
| S2 | TGX1835-10F | S24 | NGRC06815 | S46 | Chitwan-9 | S68 | F778817 |
| S3 | NGRC02691 | S25 | NGRC02699 | S47 | NGRC02684 | S69 | Surkhet-2 |
| S4 | TGX1987-62F | S26 | NGRC02664 | S48 | NGRC06813 | S70 | 2003KS-TB1xKB-5.37 |
| S5 | Soy Agd-011-2 | S27 | NGRC02707 | S49 | TGX1987-42F | S71 | 2003KS-TB1xKB-5.39 |
| S6 | Solu coll-2-2016 | S28 | NGRC05101 | S50 | Soy Agd-001 | S72 | Soy Agd-002 |
| S7 | NGRC06826 | S29 | 2003KS-TB1xKB-5.66 | S51 | Soy Agd-006 | S73 | TGX1876-4E |

| Lab code | Genotype Name | Lab code | Genotype Name | Lab code | Genotype Name | Lab code | Genotype Name |
|---|---|---|---|---|---|---|---|
| S8 | NGRC08244 | S30 | NGRC06830 | S52 | G8754 | S74 | Soy Agd-008 |
| S9 | 2003KS-KBxTB1-2.1-3 | S31 | AGS 377 | S53 | NGRC06812 | S75 | 2003KS-TB1xKB-5.62 |
| S10 | Seti | S32 | NGRC08245 | S54 | Soy Agd-021 | S76 | TGX1485-ID |
| S11 | 2003KS-TB1xKB-5.14-2 | S33 | 010-10.2 | S55 | NGRC07369 | S77 | NGRC06816 |
| S12 | Sindhuli Khairo | S34 | NGRC02690 | S56 | TGX311-23D | S78 | Soy Agd-020 |
| S13 | NGRC02676 | S35 | IARS-87-1 | S57 | NGRC07367 | S79 | NGRC06823 |
| S14 | Bringi,Pyuthan Bazar-2016 | S36 | NGRC02686 | S58 | Palpa | S80 | 2003KS-TB1xKB-5.67 |
| S15 | Lumle Bhatmas 1 | S37 | TGX1987-11E | S59 | NGRC07368 | S81 | Brown, Jumla-2016 |
| S16 | NGRC02716 | S38 | TGX 1925-1F | S60 | Soy Agd-010 | S82 | NGRC02674 |
| S17 | AGS 377 | S39 | AGS-376 | S61 | Soy Agd-014 | S83 | TGX1990-8F |
| S18 | Soy Agd-013 | S40 | NGRC06835 | S62 | Ransom | S84 | G-8586 |
| S19 | NGRC02711 | S41 | NGRC06822 | S63 | Puja | S85 | NGRC02717 |
| S20 | 2003KS-TB1xKB-5.45 | S42 | Tanahu-Creami | S64 | 2003KS-TB1xKB-5.69 | S86 | NGRC06833 |
| S21 | NGRC06809 | S43 | GC8234GC-13 | S65 | 200525(Rampur) | S87 | 2003KS-TB1xKB-5.32-2 |
| S22 | Solu-Small seed | S44 | Soy Agd-005 | S66 | NGRC02679 | S88 | NGRC02710 |

## DNA Extraction

Using a mortar and pestle, 1g sample of fresh leaves on liquid nitrogen was ground. A 700 µl extraction buffer containing 2% CTAB (w/v), Tris HCL pH 8.0 (0.1M), EDTA pH 8.0 (0.5M), NaCl (1.4M), 2% PVP (w/v), and 1% β-mercaptoethanol was immediately added to the pulverized paste and it had been transferred to micro-centrifuge tubes. The tubes were centrifuged at 15,000 rpm for 15 minutes after being incubated at 65 $^0$C in a water bath for an hour. Carefully transferring the aqueous phase into new tubes, an equal amount of chloroform:isoamyl alcohol (24:1) was added, and the mixture was well mixed by inversion for a few minutes. At 25 $^0$C, the mixture was centrifuged for 15 minutes at 15,000 rpm. The supernatant was placed into a fresh tube after phase separation. To improve the DNA's purity, 0.2 ml of sodium acetate was added to the supernatant. Isopropanol was added in an equal amount to each tube to precipitate the DNA. The tubes were centrifuged at 15000 rpm for 7 minutes after being maintained at -20 °C for 30 minutes to check for precipitation. The pellets were then twice washed with 96% and 70% ethanol, respectively, after the supernatant was removed. The pellets were resuspended in 1X TE buffer (Tris-HCl 10 mM, EDTA 1 mM, pH 8.0) after being air-dried.

## PCR Amplification

The reaction were carried out in Mygene L series thermo cycler (Long-Gene scientific instrument co .LTD) with respective primers stated in (**Table** 2). The reaction contains about 50-60 ng of template DNA, 2x master mix (Promega Corporation, USA ), 0.5 µm of single Primer (Macrogene Inc. , South Korea), with additional 1M MgCl2(Himedia laboratories Pvt. Ltd, India), 0.2mM dNTP Mix (Promega Corporation, USA),0.2U of T*aq* polymerase (Promega Corporation, USA). The thermo cycler was programmed for an initial denaturation step of 4 min at 94 ℃, followed by 35 cycles of respective annealing temperature (**Table** 2) for 50 sec, extension was carried out at 72 ℃ for 80 sec and final extension at 72 ℃ for 7 min and hold temperature of 4 ℃ at the end.

### Gel Eletrophoresis

The nature of separated DNA was likewise evaluated by utilizing 0.8% agarose gel in 1X TAE (50X TAE; 242gm Tris-base, 57.1 ml acidic corrosive (or 100 percent frosty corrosive) and 100ml of 0.5 M EDTA (pH-8.0) at 70V for 1 hr. Amplified PCR products were investigated utilizing 1.5% agarose gel at 80V for 2hr utilizing a similar buffer system. The gel was stained with ethidium bromide and captured utilizing a Gel Documentation framework (VWR®Genosmart 2, UK) and 100 bp laddar (Thermo Scientific), was utilized as a marker for the size correlation of the fragments.

## RESULTS

Most of Nepalese genotypes showed protein and oil traits genes as defined by marker analysis, where some of genotypes showed no amplification with respect to linking genes used. From the pool of 20 markes, five markers with having good banding pattern and easy amplification with desired size, with respect to the linked gene were selected for our study. The genotypes Soy Agd-005 (S44), NGRC02684 (S47),NGRC06813 (S48),TGX1987-42F (S49) showed amplification on all the marker system linked to both protein and oil content traits (**Table** 3). A total of 66 genotypes was affirmative for Gm-14 gene (linked to protein trait) in soybean, whereas promising results were showed by genes Gm-19 on 6 accessions, Gm-15 on 86 accessions , Gm-16 on 81 accessions, Gm-05 on 80 accessions linked to oil traits.Good amplification of Gm-05 gene at 261bp (**Figure** 1) showed the presence of oil trait gene in the genotypes by marker analysis, which can further be useful in selecting desired genotypes for research and other quality assessment experiments.

**Table 1. SSR markers used for Protein and Oil content trait in Soybean genepool.**

| Marker | Linked Gene | Trait linked to | Repeat Motif | Forward (5'……3') | Reverse (5'……3') | Tm (℃) | Product Size bp | References |
|---|---|---|---|---|---|---|---|---|
| Satt556 | Gm14 | Protein | (AAT)14 | GCGATAAAACCCGATAAATAA | GCGTTGTGCACCTTGTTTTCT | 54 | 165 | Shi et al, 2010 |
| Satt006 | Gm19 | Oil | (TAT)11 | CAATGTGATTAGTTTTGGAAA | GGGTTAATGTTGTTTTTTATA | 55 | 3141 | Rodrigues et al, 2016 |
| Satt212 | Gm15 | Oil | (TAA)9 | CCAATCCAAACAAATCCACT | CAGCAATGATGATAATGAATGA | 54 | 144/327 | Rodrigues et al, 2016 |
| Satt144 | Gm16 | Oil | (TAA)18 | CGTCGCCATCACTATGAGAA | CCATCTTGAGCAGAGTTTGAAGTT | 54 | 190 | Rodrigues et al, 2016 |
| Satt449 | Gm05 | Oil | (TTA)21 | GCGTGCTTCTTATATTAGGTGTTAGT | GCGCATTGGAGTTTTTGCTTTT | 55 | 261 | Rodrigues et al, 2016 |

**Table 1. Nepalease soybean with protein and oil content traits identified using different molecular markers**

| Linked Gene | Genotypes |
|---|---|
| **Gm14** | TGX1835-10F, NGRC02691, TGX1987-62F, Soy Agd-011-2, Solu coll-2-2016, NGRC06826, NGRC08244, 2003KS-KBxTB1-2.1-3, Seti, 2003KS-TB1xKB-5.14-2, Sindhuli Khairo, LumleBhatmas 1, NGRC02716, Soy Agd-013, NGRC02711, 2003KS-TB1xKB-5.45, NGRC06809, Solu-Small seed,NGRC06815NGRC02664, NGRC02707, NGRC05101, 2003KS-TB1xKB-5.66, NGRC06830, AGS 377, NGRC08245, 010-10.2, NGRC02690, NGRC02686, AGS-376, NGRC06835, NGRC06822, Tanahu-Creami, GC8234GC-13, Soy Agd-005, NGRC02684, NGRC06813, TGX1987-42F,G8754, NGRC06812, NGRC07369, NGRC07367, Palpa, NGRC07368, Soy Agd-010, Soy Agd-014, 200525(Rampur), NGRC02679, F778817, Surkhet-2, 2003KS-TB1xKB-5.37, 2003KS-TB1xKB-5.39, Soy Agd-002, TGX1876-4E, Soy Agd-008 ,NGRC06816, Soy Agd-020,NGRC06823, 2003KS-TB1xKB-5.67, Brown, Jumla-2016, NGRC02674, TGX1990-8F, G-8586 NGRC02717, NGRC06833, 2003KS-TB1xKB-5.32-2 . |
| **Gm19** | Soy Agd-005, 2003KS-TB1xKB-5.4, Chitwan-9, NGRC02684, NGRC06813, TGX1987-42F |
| **Gm15** | Soy Agd-005, 2003KS-TB1xKB-5.4, Chitwan-9, NGRC02684, NGRC06813, TGX1987-42F,NGRC02687, TGX1835-10F, NGRC02691, TGX1987-62F, Soy Agd-011-2, Solu coll-2-2016, NGRC06826, NGRC08244, 2003KS-KBxTB1-2.1-3, Seti, 2003KS-TB1xKB-5.14-2, Sindhuli Khairo, NGRC02676, Bringi,Pyuthan Bazar-2016, LumleBhatmas 1, NGRC02716, AGS 377, Soy Agd-013, NGRC02711, 2003KS-TB1xKB-5.45, NGRC06809, Solu-Small seed, NGRC02703, NGRC06815, NGRC02699, NGRC02664, NGRC02707, NGRC05101, 2003KS-TB1xKB-5.66, NGRC06830, AGS 377, NGRC08245, 010-10.2 ,NGRC02690, IARS-87-1, NGRC02686, TGX1987-11E, TGX 1925-1F,AGS-376, NGRC06835, NGRC06822, Tanahu-Creami, GC8234GC-13, Soy Agd-005, 2003KS-TB1xKB-5.4, Chitwan-9, NGRC02684, NGRC06813, Soy Agd-001, Soy Agd-006, G8754, GRC06812, Soy Agd-021, NGRC07369, TGX311-23D, NGRC07367, Palpa,NGRC07368 Soy Agd-010, Soy Agd-014, Ransom Puja, 2003KS-TB1xKB-5.69, NGRC02679, NGRC02672, F778817 Surkhet-2, 2003KS-TB1xKB-5.37, 2003KS-TB1xKB-5.39, Soy Agd-002, TGX1876-4E, Soy Agd-008, 2003KS-TB1xKB-5.62, TGX1485-ID, NGRC06816, Soy Agd-020,NGRC06823, 2003KS-TB1xKB-5.67, Brown, Jumla-2016, NGRC02674, TGX1990-8F, G-8586, NGRC02717, NGRC06833, 2003KS-TB1xKB-5.32-2, NGRC02710. |
| **Gm 16** | NGRC02687, TGX1835-10F ,NGRC02691, TGX1987-62F, Soy Agd-011-2, Solu coll-2-2016, NGRC06826, NGRC08244, 2003KS-KBxTB1-2.1-3, Seti, 2003KS-TB1xKB-5.14-2, Sindhuli Khairo, NGRC02676 ,Bringi,Pyuthan Bazar-2016, LumleBhatmas 1, NGRC02716, AGS 377, Soy Agd-013, NGRC02711, 2003KS-TB1xKB-5.45, NGRC06809 ,Solu-Small seed, NGRC02703 ,NGRC06815, NGRC02699,NGRC02664, NGRC02707, NGRC05101, 2003KS-TB1xKB-5.66, NGRC06830, AGS 377, NGRC08245, 010-10.2 ,NGRC02690, IARS-87-1, NGRC02686, TGX1987-11E, TGX 1925-1F,AGS-376, NGRC06835, NGRC06822, Tanahu-Creami, GC8234GC-13, Soy Agd-005, 2003KS-TB1xKB-5.4, Chitwan-9, NGRC02684, NGRC06813, Soy Agd-001, Soy Agd-006, G8754, NGRC06812, Soy Agd-021, NGRC07369, TGX311-23D, NGRC07367, Palpa,NGRC07368, Soy Agd-010, Soy Agd-014, Ransom Puja, 2003KS-TB1xKB-5.69, NGRC02679, NGRC02672, F778817, Surkhet-2, 2003KS-TB1xKB-5.37, 2003KS-TB1xKB-5.39, Soy Agd-002, TGX1876-4E, Soy Agd-008, 2003KS-TB1xKB-5.62, TGX1485-ID, NGRC06816, Soy Agd-020, NGRC06823, 2003KS-TB1xKB-5.67, Brown, Jumla-2016, NGRC02674, TGX1990-8F, G-8586 ,NGRC02717, NGRC06833, 2003KS-TB1xKB-5.32-2 ,NGRC02710 . |
| **Gm 05** | NGRC02687, NGRC02691, TGX1987-62F, Soy Agd-011-2, Solu coll-2-2016, NGRC06826, 2003KS-KBxTB1-2.1-3, Seti, 2003KS-TB1xKB-5.14-2, Sindhuli Khairo ,NGRC02676, Bringi,Pyuthan Bazar-2016, LumleBhatmas 1, NGRC02716, Soy Agd-013 ,NGRC02711, 2003KS-TB1xKB-5.45, NGRC06809, Solu-Small seed, NGRC02703, NGRC06815, NGRC02699, NGRC02664, NGRC02707, NGRC05101, 2003KS-TB1xKB-5.66, NGRC06830, AGS 377, NGRC08245, 010-10.2, NGRC02690, IARS-87-1, NGRC02686, TGX1987-11E, AGS-376, NGRC06835, NGRC06822, Tanahu-Creami,GC8234GC-13, Soy Agd-005, 2003KS-TB1xKB-5.4, Chitwan-9, NGRC02684, TGX1987-42F, Soy Agd-001, Soy Agd-006, G8754 NGRC06812, Soy Agd-021, NGRC07369, TGX311-23D, NGRC07367, Palpa, NGRC07368, Soy Agd-010, Soy Agd-014, Ransom Puja, 2003KS-TB1xKB-5.69, 200525(Rampur), NGRC02679, NGRC02672, F778817, Surkhet-2 ,2003KS-TB1xKB-5.37, 2003KS-TB1xKB-5.39, Soy Agd-002, TGX1876-4E, Soy Agd-008, 2003KS-TB1xKB-5.62, TGX1485-ID, NGRC06816, Soy Agd-020, NGRC06823, 2003KS-TB1xKB-5.67, Brown, Jumla-2016,NGRC02674, TGX1990-8F, G-8586, 2003KS-TB1xKB-5.32-2. |

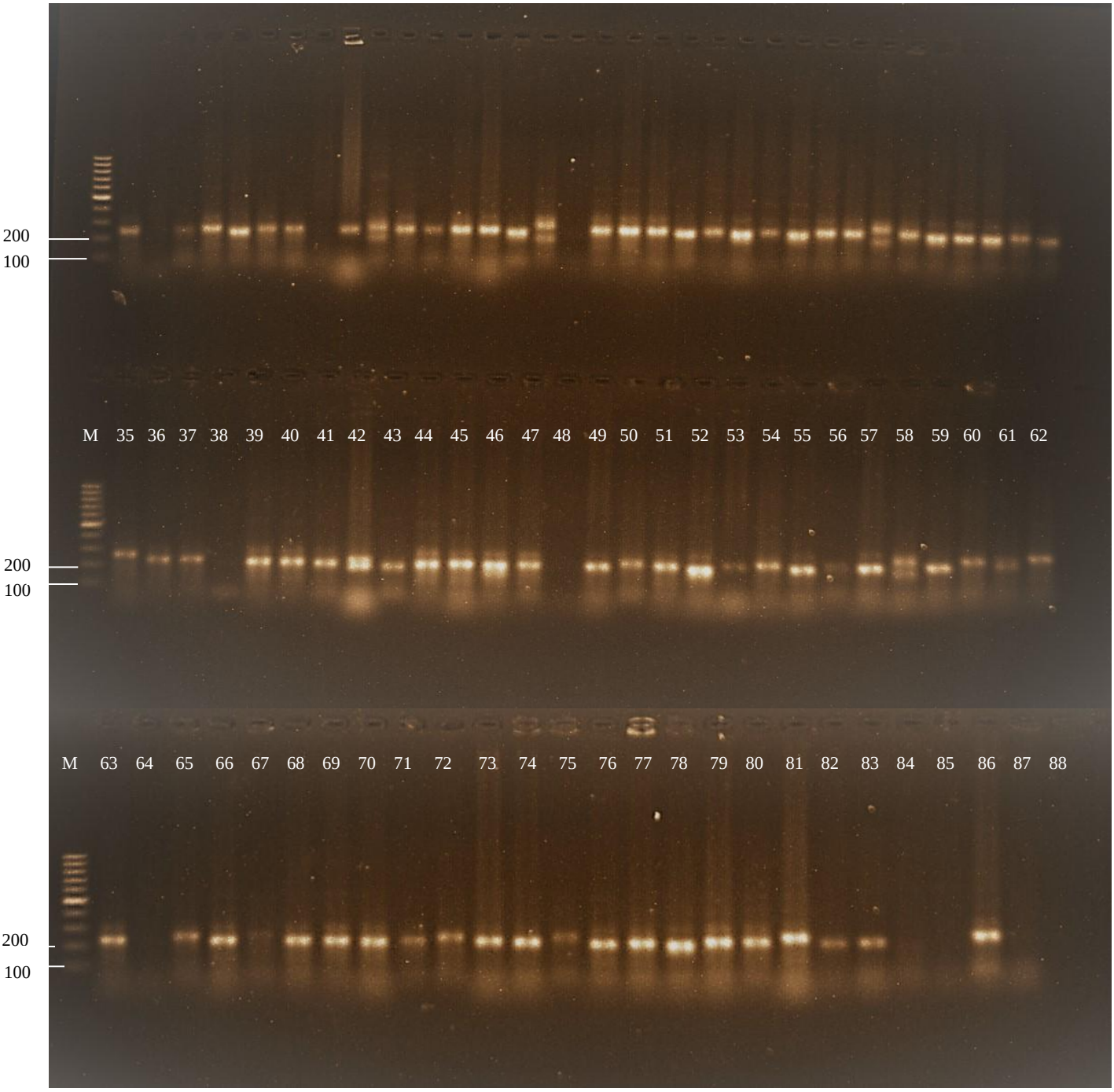


**Figure 1: M represents DNA ladder of 100 bp . PCR Amplification gene Gm-05 (261bp) respective to oil trait across 88 genotype (Table 1).**

## DISCUSSIONS

We documented soybean traits linked to good oil and protein genes for the 88 genotypes from Nepal and acts as a base for further quality selection of genotypes in accord to the traits and diversity analysis. The screening of the genotypes by the use of molecular markers before the phenotypic study saves both time and money. The use of SSR markers as a trait screening by QTL analysis has been reported and implemented in many breeding programs. Hyten etal (2004) reported the use of SSR markers associated to oil content in food grade soybean to be used in MAS breeding. The SSR markers identified provide a

flexibility for MAS breeding involving both protein and oil selections (Shi et al 2010). SSR markers also were employed in a MAS program to create high-yielding soybean varieties with resistance to soybean mosaic virus(Liu et al 2018).Due to incomplete, unavailable, or inadequately detailed information regarding access genealogy and the lack of environmental influence on molecular markers relative to the majority of agronomic traits, molecular markers have been the most preferred methodologies used to assess genetic relationships between cultivars (Mulato et al2010). From our study, the 88 genotypes were assessed accordingly to the linked genes. The result shows promising data which relates to the presence of the genes. Conventionally, the breeding would take up time and effort on screening off the genotypes for its traits. This study provides a baseline on selecting the genotypes for its protein and oil triats wich can be useful in further breeding and commercializing of choosen genotypes. The results gives some insights to the use of markers in selecting of genotypes, not necessarlily giving spot on data. Some of the markers were able to distinguish the trait location but still extensive research on trait location and its co-relation to the phenotype data must be carried out using diverse set of markers. It is not possible to definitively say if these qualities from molecular analysis done in the study connect with the physical traits in the field, thus additional research with more robust markers and substantial data gathering is required to validate the analysis done.Nevertheless, the study can be extended using more marker and diverse genotypes with respective traits in screening good genotype for breeding.

## CONCLUSION

The study can serve as a starting point for the evaluation of soybeans using attributes and genes associated with those traits. We can use the idea of MAS breeding and diversity on soybean germplasm for its enhancement and sustainable production in relation to food quality using this molecular method. From the study, we can further use refine the breeding methods subsequently focusing on selecting the best genotypes showing positive towards the protein and oil genes. By using the MAS breeding strategy, it is possible to improve soybeans by choosing the genotype with the necessary features and subsequently conserving resources in an effort to increase output and fulfill market demands.

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