



Oil content and fatty acid composition of soybean (*Glycine max* L.) genotypes evaluated under rainfed conditions of Kashmir Himalayas in India

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Received: May 14, 2015; Revised received: October 10, 2015; Accepted: November 8, 2015

Abstract: Soybean (*Glycine max*), a multipurpose crop with much appreciated nutritional and functional properties, has generated a lot of interest during last few decades for improving its cultivation and characteristics. Besides having a great potential of energy-protein malnutrition eradication, it can contribute greatly to edible oil pool in the region. This study investigates some agro-morphological characters, besides seed oil content and fatty acid composition of two local soybean genotypes labeled as S/D-18 and S/D-22 grown under rainfed conditions of Kashmir Himalayas in India. The genotype S/D-22 matured earlier than that of S/D-18. Seed yield/plant of 18.5 g (S/D-18) and 20.1g (S/D-22) was recorded in these genotypes with 100-seed weight of 14.3 g and 14.7 g respectively. Seeds of genotype S/D-18 yielded an oil content of 19.54% while in genotype S/D-22 oil content of 19.74% was recorded. Fatty acids of myristic, palmitic, stearic, oleic, linoleic and linoleinic were detected and quantified in the oil extracted from the seeds of these genotypes. Saturated fatty acids constituted 11.88%(S/D-18) and 11.42%(S/D-22) while unsaturated fatty acids constituted 88.12% (S/D-18) and 88.58% (S/D-22) of the oil. The study has thus, revealed that under dry land conditions, although soybean seed yield/plant is lesser, percentage oil content and fatty acid composition essentially remained unaltered and that significant differences can occur in individual fatty acid contents between genotypes.

Keywords: Evaluation, Fatty acids, Gas chromatography, Oil content, Soybean genotypes

INTRODUCTION

The soybean [*Glycine max* (L.) Merrill, family Leguminosae] classified as an oilseed rather than a pulse by UN Food and Agricultural Organization (FAO) is of worldwide interest (Cheng *et al.*, 2008; Lee *et al.*, 2008). It is one of the most important agricultural crops for oil and protein and has a natural capacity to fix nitrogen from the atmosphere (Singh and Hymowitz, 1999). It has been the dominant oilseed since the 1960s besides, containing 40% protein unlike most other beans that contain only about 20% protein (Greenberg and Hartung, 1998). Oil content in dry soybean seeds on an average is 19% and can vary from 6.5% to 28.7% depending on the plant genetic background and growth conditions (Weselake *et al.*, 2009). Soybean is a multipurpose crop used as human food, livestock feed, for industrial purposes, and more recently, as a source of bio-energy (Myaka *et al.*, 2005). The domestication of *Glycine max* from the wild species (*Glycine soja* Sieb. and Zucc.) occurred in China but there is no consensus about the location within China (Carter *et al.*, 2004). *Glycine max* differs from *G. soja* with smaller pods and seeds, stems that

are viny and twining, and nearly complete shattering at plant maturity. The cultivated landraces were initially introduced into Korea and later into Japan some 2000 years ago (Kihara, 1969) and then distributed throughout Asia and more recently extended to Europe and America (Tavaux-Pirra *et al.*, 2009). Soybeans thus have been grown as a food crop for thousands of years in China and other countries of East and South East Asia and constitute to this day, an important component of the traditional popular diet in these regions. Despite its economic importance as crop for feed, oil and soy food products, the genetic base of soybean cultivars is extremely narrow. The indigenous cultivars and landraces in East Asia are on the verge of extinction because farmers are now growing high yielding soybean cultivars (Singh and Hymowitz, 1999). Soybean is grown in around 50 countries of the world (Boerma and Specht, 2004) and its production has reached 249 million metric tons (mmt) in 2014. Brazil (90.0 mmt), United States (89.5 mmt), Argentina (52.6 mmt), China (15.0 mmt) and India (9.8 mmt) are the leading producers (FAO, 2014). Despite the relatively low oil content of the seed (about 20% on

moisture-free basis), soybeans are the largest single source of edible oil and account for roughly 50% of the total oilseed production of the world.

In India it is believed that soybean came centuries ago probably either from China through the Himalayan mountains or from Indonesia via Burma (Myanmar) (Singh, 2006). Until 1970 its cultivation was negligible in the country however, during last 45 years especially since 1990 there has been a tremendous increase in the area under its cultivation besides production supplementing about 25% of edible oil produced in India, thus playing a very important role in resolving edible oil deficit situation (Sharma *et al.*, 2014). The production of soybean in India (4% of global) at present is restricted mainly to states of Madhya Pradesh, Uttar Pradesh, Maharashtra and Gujarat. It is also grown on small scale acreage in Himachal Pradesh, Punjab and Delhi (Singh, 2006). In 1940s and 50s the North-Western Indian Himalayan state of Jammu and Kashmir was leading in production of soybeans; 502 tons on 1,757 acres (Shurtleff and Aoyagi, 2012). Presently however as compared to other Indian states, soybean cultivation and production in Jammu and Kashmir is negligible. It is raised on a small scale for domestic use either pure or in mixture with maize. The tradition of sowing soybean on paddy field bunds is still followed at some places in the state although this practice was much more common few decades back and is supposed to give some protection to paddy field bunds besides yielding few kilograms of seed for household use. Roasted soybean seeds frequently sold by street vendors across the state have been a popular snack of local

Table 1. Average monthly temperature and rainfall recorded during the soybean growing season in Srinagar, Jammu and Kashmir (India).

Month	Temperature (°C)		Rainfall (mm)
	Minimum	Maximum	
April	8	19	99
May	11	24	53
June	15	29	17
July	19	30	47
August	17	29	71
September	13	25	120
October	9	22	39

Table 2. Agro-morphological traits of 2 soybean (*Glycine max*) genotypes evaluated under rainfed conditions of Kashmir Himalayas.

Agro-morphological trait	Genotype	
	S/D-18	S/D-22
Plant height (cms)	79.5±4.6	80.5±5.0
Days to maturity	151.0±2.6 ^b	140.0±1.5
Number of pods/plant	102.0±8.7	113.3±19.6 ^b
Number of seeds/pod	1.4±0.4	1.5±0.6
Seed yield/plant (g)	18.5±3.3	20.1±3.9 ^b
100-seed weight (g)	14.3±0.7	14.7±0.5

Values are mean± SD; ^bMeans are significantly higher at 5% level of confidence

people especially during harsh winters. The indigenous black soybeans grown mostly in the Himalayan range especially in Kumau and Garwal region are also cultivated in some parts of Jammu and Kashmir and often used as livestock feed during winters. Considering the potential of soybean towards contributing to edible oil pool and energy-protein malnutrition eradication together with current prices and global production trends, there is much scope and therefore a strong need for promotion of sustainable soybean production in the state of Jammu and Kashmir. Data on oil content and fatty acid composition of local soybean germplasm are limited. Such studies are necessary for proper utilization of the crop. The main aim of present study was therefore, to determine oil content and investigate fatty acid composition in local soybean genotypes evaluated under dry land conditions of Kashmir Himalayas.

MATERIALS AND METHODS

Plant material and growing conditions: The seeds of the two genotypes of soybean [*Glycine max* (L.) Merrill] labeled as S/D-18 and S/D-22 were collected in 2013 from hills in Doda district of Jammu province of North-Western Indian Himalayan state of Jammu and Kashmir (33°17' – 37°20' N latitude, 73°25' – 80° 30' E longitude). The experiment was conducted during summer (*Kharif*) season in 2014 under rainfed conditions at the Experimental Farm of National Bureau of Plant Genetic Resources, Regional Station Srinagar, Jammu and Kashmir (33°59' N latitude, 74°47' E longitude, 1639 m above sea level). The experimental site is located in dryland area falling within temperate climatic zone. The seeds were sown in rows in the beds which were three (03) meters wide; the individual rows were 40 centimeter apart. Ten rows with a minimum of seven plants/row were maintained for each genotype. The average monthly temperatures and rainfall during the study period are shown in Table 1. Physical and chemical properties of the soil were determined before the start of experiment. The soil was having clay, silt and sand in the proportion of

Table 3. Oil content and fatty acid composition of 2 soybean (*Glycine max*) genotypes evaluated under rainfed conditions of Kashmir Himalayas.

	Genotype	
	S/D-18	S/D-22
Total oil content (%)	19.54±0.017	19.74±0.104
Fatty acid content (%)		
Myristic acid	0.065±0.0004 ^b	0.058±0.0005
Palmitic acid	9.79±0.00021	0.20±0.0007
Stearic acid	2.02±0.0035 ^b	1.15±0.0052
Oleic acid	20.09±0.0035	20.88±0.0017
Linoleic acid	56.79±0.0017	57.91±0.0035 ^b
Linolenic acid	11.24±0.0052 ^b	9.79±0.0104

Values are mean of three replicates ±SD; ^b Means are significantly higher at 5% level of confidence



Fig. 1. Soybean (*Glycine max*) genotypes S/D-22 and S/D-18 evaluated in the present study.

33.4:60:6.6 with organic carbon content of 1.02%, electrical conductivity of 0.133 mhos/cm and pH of 6.23. Available nitrogen, phosphorus and potassium content in the soil was 322.54 ppm, 17.57 ppm and 451.63 ppm respectively.

Agro-morphological characterization: Ten plants of each genotype were randomly sampled for recording plant height (cm), number of pods/plant and seed yield/plant (g). 100-seed weight was determined in three independent samples from pooled grain yield. Plants growing on the peripheries of the experimental plot were not considered for sampling.

Determination of oil content and fatty acid profile: Once harvested, seeds were cleaned and dried to approximately 7% moisture. Seed oil content in three samples from each genotype was determined by a wide line nuclear magnetic resonance (NMR) method according to Robertson and Morrison (1979).

The concentration of fatty acids was measured as their corresponding methyl esters. The preparation of methyl esters of fatty acids (FAMES) was carried out according to the International Standard ISO 5509:2000 – boron trifluoride (BF₃) method (EN ISO 5509:2000). 5-10 seeds were crushed with the help of mortar and pestle and 1.5-2.0 g of this crushed material was taken in test tubes. After adding 3 ml petroleum ether the tubes were covered with teflon and kept overnight. 2 ml 0.5N NaOH prepared in methanol were added and mixed gently and the tubes were placed in hot water bath at 90-97°C for 5 min. Afterwards, 2 ml boron trifluoride (BF₃) was added and contents were stirred and tubes again placed in water bath at 97°C for 5 min. Then 3 ml petroleum ether and 2 ml deionized water were added followed by stirring. Two layers got separated as the tubes were left undisturbed for one hour at room temperature. From upper layer 1 ml sample was taken in GC vials for analysis. Analysis was carried out using Gas Chromatograph (GC) Model-14B, Shi-

madzu, Japan equipped with SP-2340 flexible fused silica capillary column (60m x 0.25mm x 0.2 μm film thickness) and a flame ionization detector. Nitrogen was used as a carrier gas with flow rate of 20 ml/min, and the column oven was set to 185°C, and the injector and detector were set to 200°C. The relative percentage (area %) of the fatty acids was determined using a reference mixture of methyl esters of fatty acids. The analyses were carried out in triplicate.

Statistical analysis: Student's *t*-test (Microsoft Excel) was used to assess the significance of differences between mean values. Differences were considered significant for *p* values < 0.05.

RESULTS AND DISCUSSION

The mean values of agro-morphological traits recorded during our present study are given in Table 2. While plant height is almost same in the two genotypes there is a significant difference in days to maturity; yellowing of leaves in genotype S/D-22 occurred earlier maturing 11 days earlier than that of S/D-18 (Fig. 1). Significantly higher number of pods/plant has also been recorded in genotype S/D-22 as compared to genotype S/D-18 with almost same number of seeds/pod in both the genotypes. Compared to plant height there was a greater variation in number of pods among individual plants. Majority of the pods in both genotypes were having one or two rarely three seeds. Seeds in these genotypes are yellowish or light yellow in color and more or less spherical or oval shaped. In genotype S/D-22 comparatively higher seed yield/plant and 100-seed weight has been recorded than in genotype S/D-18.

The oil content and fatty acid composition of these soybean genotypes has been presented in Table 3. Higher oil content of 19.74% has been recorded in genotype S/D-22 than in S/D-18 (19.54%). The fatty acids detected in the oil extracted from seeds of the two genotypes during present study are myristic, palmitic, stearic, oleic, linoleic and linoleic (Fig. 2). In the oil obtained from seeds of genotype S/D-18, significantly higher contents of myristic, stearic and linoleic acids were recorded compared to S/D-22 whereas in latter genotype comparatively higher contents of palmitic, oleic and linoleic acids were recorded than in S/D-18, the difference was significant only in case of linoleic acid. Our study has thus revealed that saturated fatty acids constitute 11.88% (S/D-18) and 11.42% (S/D-22) while unsaturated fatty acids constitute 88.12% (S/D-18) and 88.58% (S/D-22) of the oil. Soybeans grow well in almost all types of soil except sandy soils with very poor water retention, growing best in temperate climates. Rainfall, temperature and photoperiod during the growing season are determinant environmental factors affecting grain yield. Drought particularly in the reproductive growth stage (Oya *et al.*, 2004) is the most important abiotic stress limiting soybean yield (Specht *et al.*, 1999; Purcell and Specht,

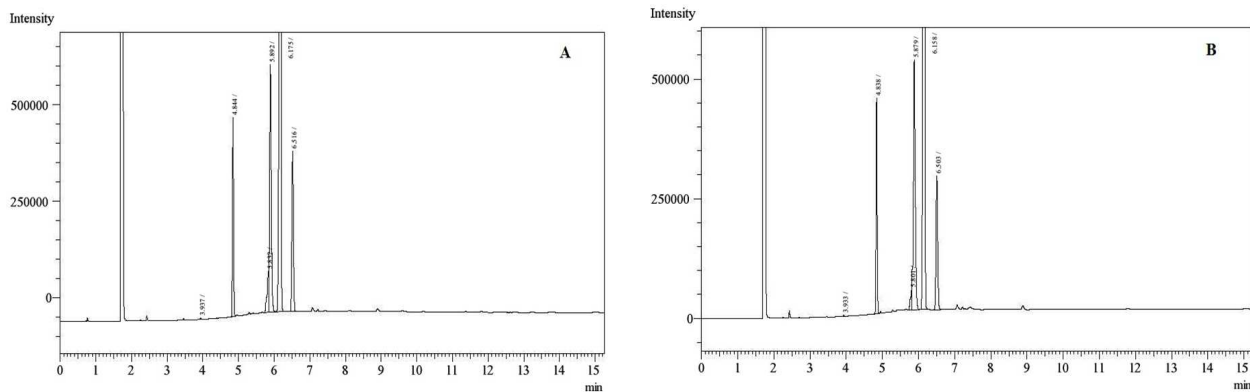


Fig. 2. Gas chromatograms showing separation of FAMES in the oil extracted from seeds of two soybean (*Glycine max*) genotypes S/D-18(A) and S/D-22(B).

2004). Rainfall in the range of 500 to 700 mm is required for good yields. Adequate water supply is especially important during the period of pod and seed development. Grain yield is one of the most important selection criteria used by breeders. It is influenced by different yield components that include number of pods and seeds per plant as well as weight of 100 seeds (Rotzler *et al.*, 2009). Seed weight of commercially grown soybeans are generally 10 - 20 g/100 seeds but can be larger than 30 g/100 seeds or smaller than 6 g/100 seeds (Lersten and Carlson, 2004). Lesser number of seeds/pod recorded during the course of present study compared to normal average of 2.5 seeds and consequently lower yield/plant can be attributed to stressful conditions mostly due to inadequate or untimely rainfall. Slow wilting and minimal yield reduction under drought are important traits in evaluating drought tolerance. Genetic improvement for drought tolerance therefore, is an effective method to reduce yield loss in soybean (Pathan *et al.*, 2014). It is a proven fact that narrow row spacing is a risk management tool that helps to stabilize soybean yields in stressful environments, that is why row spacing in present experimentation was kept only 40 cms. Narrow rows have a yield advantage because they achieve canopy closure more quickly and intercept more light throughout the growing season besides preventing moisture loss from the soil. Being a short-day plant soybean flowers when the nights begin to lengthen. Soybean plants flower earlier when daylight period is short. That is why plants flowered during the months of June and July in the present study although seeds were sown in the last week of April.

Oil content can vary from 6.5% to 28.7% in dry soybean seeds depending on the plant genetic background and growth conditions (Weselake *et al.*, 2009). Typically they contain about 20% oil on a dry weight basis (Wilson, 2004) and five fatty acids make up nearly the entire oil portion of soybean seed. Soybean oil averages 12% palmitic acid (16:0), 4% stearic acid (18:0), 23% oleic acid (18:1), 53% linoleic acid (18:2), and 8% linolenic acid (18:3). The 16:0 and 18:0 fractions are saturated fatty acids and constitute 15% of the

soybean oil. The remainder of the oil (about 85%) is made up of unsaturated fatty acids or 18:1, 18:2, and 18:3 (Lee *et al.*, 2007). It has also been reported in a recent study that proportion of linoleic acid in soybean oil ranged from 49.0% to 53.5%, oleic acid from 21.4% to 26.6% while palmitic acid was found to be in the range of 9.2% to 11.2% (Matthaus and Ozcan, 2014). In yet another more recent study linoleic, linolenic and stearic acids were reported to constitute 50.20%, 7.75% and 3.69% of soybean oil respectively (Mohammadi, 2015). Our results therefore almost corroborate with the earlier reports. It is because of high percentage of polyunsaturated fatty acids (18:2 and 18:3) that soybeans are considered to be unstable (Neff and List, 1999). Fatty acid composition is a major determinant of oil quality in oilseeds and is therefore, an important consideration for breeding programs (Daun, 1998). Proper levels of unsaturated fatty acid moieties in vegetable oils have been recognized as good nutritional characteristics for health. One goal of breeding projects is the alteration of the fatty acid composition, for example, the reduction of the level of linoleic acid moiety. The ideal oil with the most end uses would have saturates (palmitic + stearic acids) reduced from 15 to < 7%, oleic acid increased from 23 to > 55%, and linolenic acid reduced from 8 to < 3% (Lee *et al.*, 2007). Genetic sources are very important in improving soybean oil content although, genotype x environmental interactions which influence the oil concentration and fatty acid profile of soybean oil have been addressed in many studies. Moisture stress effects on yield, seed weight, oil and protein contents of seeds were evaluated in dry land and irrigated plots and 57-68% of the seed weight loss was attributed to changes in weight per seed of oil and protein (Rose, 1988). When compared to the irrigated treatments, the oil and protein percentages of the dry land seed varied. Studies have indicated that temperature plays an important role in the synthesis of oil and fatty acids. In general, higher temperature increases oil content in soybean seed (Wilson, 2004). Soybeans grown under high average temperatures have reduced linoleic acid and linolenic acid, and increased oleic acid content; however,

contents of saturated fatty acids were changed little by environment (Wilson, 2004; Hou *et al.*, 2006). Our study has revealed that under dry land conditions, although soybean seed yield/plant is lesser, %age oil content and fatty acid composition essentially remained unaltered and that saturated fatty acid content in the two genotypes studied was around 11%, value below the normal average of 15% suggesting the possibility of occurrence of soybean genotypes with such desired traits in the state of Jammu and Kashmir. Soybean, thus has a great potential to be exploited as an edible oilseed crop under dry land conditions throughout this Indian Himalayan state utilizing proper management practices. In absence of adequate rainfall, however, irrigation will always prove to be an essential factor for increased profit and security to the farmer.

Conclusion

Our study has revealed that under dry land conditions, although soybean seed yield/plant is lesser, percentage oil content and fatty acid composition essentially remained unaltered and that significant differences can occur in individual fatty acid contents between genotypes. Extensive survey in the entire state of Jammu and Kashmir may yield soybean genotypes with desired traits including abiotic and biotic stress tolerance. Characterization and evaluation of more soybean genotypes for such traits particularly under unfavorable environments where water stress is common and yields are low should accelerate breeding progress for promotion and exploitation of this crop as oil seed in the entire region.

ACKNOWLEDGEMENTS

The authors are thankful to Director, National Bureau of Plant Genetic Resources (NBPGR), Indian Council of Agricultural Research (ICAR), New Delhi and to Bhabha Atomic Research Center, Trombay, Maharashtra for facilities and help.

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