BIOCHEMICAL EVALUATION OF IMMATURE PODS AND MATURE SEEDS OF WILD *ABELMOSCHUS* GENOTYPES

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in
BIOCHEMISTRY
(Minor Subject: Botany)

By

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CERTIFICATE I

This is to certify that the thesis entitled, **"BIOCHEMICAL EVALUATION OF IMMATURE PODS AND MATURE SEEDS OF WILD *ABELMOSCHUS* GENOTYPES"** submitted for the degree of **Integrated Master of Science (Hons.)** in the subject of **Biochemistry** (Minor subject: **Botany**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Amandeep Kaur (L-2015-BS-27-IM)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE – II

This is to certify that the thesis entitled, **"BIOCHEMICAL EVALUATION OF IMMATURE PODS AND MATURE SEEDS OF WILD *ABELMOSCHUS* GENOTYPES"**, submitted by **Amandeep Kaur (L-2015-BS-27-IM)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Integrated Master of Science (Hons.)**, in the subject of **Biochemistry** (Minor subject: **Botany**) has been approved by the Student's Advisory Committee along with External Examiner after an oral examination on the same.

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ABSTRACT

In the present study the immature pods and seeds of wild *Abelmoschus* species were biochemically characterised. Total carbohydrates in pods varied from 22.34- 93.10% DW. Total soluble sugars (TSS), reducing sugars and non-reducing sugars exhibited range of 13.74- 84.36%, 0.17- 19.41% and 31.25- 64.95% DW respectively. Mucilage, dry matter, ash, crude protein and total soluble protein depicted range of 6.64- 19.64% DW, 11.18- 27.59%, 1.10- 6.94%, 2.13- 6.32% and 0.93- 3.56% FW respectively. Free amino acids range was 0.16- 0.27% (FW). Carotenoids, phenolics, O-dihydroxyphenols and flavonols varied from 4.48- 13.25 mg/100 g FW, 122.95- 412.01 mg/100 g DW, 4.30- 17.99 mg/100g and 63.63- 274.49 mg/100g DW respectively. Vitamin E and C varied from 15.14- 51.24 mg/100 g DW and 41.26- 120.10 mg/100 g FW respectively. DPPH and FRAP activity ranged from 39.74- 62.90% and 4.54- 11.22 mg AAE/g DW respectively. Phytate, oxalate, saponin and tannins depicted variation of 0.65- 2.08% DW, 0.23- 0.63% DW, 0.19- 1.04% DW, and 1.26- 3.30% DW respectively. Seed oil ranged from 10.68-27.61% DW. *A. manihot* and *A. tetraphyllus* pods were high in antioxidants, mucilage and low in tannins and saponins. *A. moschatus* was rich in nutrients in pods and Vit. E, oleic, linoleic acid and minerals in seeds, while *A. tetraphyllus* possessed high antioxidants and was low in phytate and oxalate and *A. mizonagenesis* exhibited high seed oil content. These genotypes can be used for improvement of cultivated okra for end product diversification.

Keywords: Okra, wild, pods, seeds, nutrients, anti-nutrients, antioxidants

Signature of Major Advisor

Signature of the Student

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ਸਾਰ ਅੰਸ਼

ਮੌਜੂਦਾ ਅਧਿਐਨ ਦੌਰਾਨ ਭਿੰਡੀ ਦੀਆਂ ਜੰਗਲੀ ਪ੍ਰਜਾਤੀਆਂ ਦੀਆਂ ਅਧੂਰੀਆਂ ਛੋਟੀਆਂ ਫ਼ਲੀਆਂ ਅਤੇ ਬੀਜਾਂ ਦਾ ਜੀਵ-ਰਸਾਇਣਕ ਮੁਲਾਂਕਣ ਕੀਤਾ ਗਿਆ। ਫ਼ਲੀਆਂ ਵਿੱਚ ਕੁੱਲ ਕਾਰਬੋਹਾਈਡ੍ਰੇਟਸ ਦੀ ਮਾਤਰਾ 22.34- 93.10% DW ਸੀ। ਕੁੱਲ ਘੁਲਣਸ਼ੀਲ ਸ਼ੂਗਰ (TSS), ਰਡਿਊਸਿੰਗ ਸ਼ੂਗਰ ਅਤੇ ਨਾਨ-ਰਡਿਊਸਿੰਗ ਸ਼ੂਗਰ ਦੀ ਮਾਤਰਾ ਕ੍ਰਮਵਾਰ 13.74- 84.36%, 0.17- 19.41% ਅਤੇ 31.25- 64.95% DW ਸੀ। ਗੋਂਦ (ਲੇਸਦਾਰ ਪਦਾਰਥ), ਸੁੱਕੇ ਮਾਦੇ, ਐਸ਼, ਕਰੂਡ ਪ੍ਰੋਟੀਨ ਅਤੇ ਕੁੱਲ ਘੁਲਣਸ਼ੀਲ ਪ੍ਰੋਟੀਨ ਦੀ ਮਾਤਰਾ ਕ੍ਰਮਵਾਰ 6.64- 19.64% DW, 11.18- 27.59%, 1.10- 6.94%, 2.13- 6.32% ਅਤੇ 0.93- 3.56% FW ਸੀ। ਫ੍ਰੀ ਐਮਾਈਨੋ ਐਸਿਡ ਦੀ ਮਾਤਰਾ 0.16-0.27% (FW) ਸੀ। ਕੈਰੋਟੀਨਾਇਡਸ, ਫਿਨੋਲਿਕਸ, ਓ-ਡਾਈਹਾਈਡ੍ਰੋਕਸੀਫਿਨੋਲਸ ਅਤੇ ਫਲੇਵੋਨੋਲਸ ਦੀ ਮਾਤਰਾ ਕ੍ਰਮਵਾਰ 4.48- 13.25 mg/100 g FW, 122.95- 412.01 mg/100 g DW, 4.30- 17.99 mg/100g ਅਤੇ 63.63- 274.49 mg/100g DW ਸੀ। ਵਿਟਾਮਿਨ ਈ ਅਤੇ ਸੀ ਦੀ ਮਾਤਰਾ ਕ੍ਰਮਵਾਰ 15.14-51.24 mg/100 g DW ਅਤੇ 41.26- 120.10 mg/100 g FW ਸੀ। DPPH ਅਤੇ FRAP ਗਤੀਵਿਧੀਆਂ ਕ੍ਰਮਵਾਰ 39.74-62.90% ਅਤੇ 4.54-11.22 mg AAE/g DW ਦਰਜ ਕੀਤੀਆਂ ਗਈਆਂ। ਫਾਈਟੇਟ, ਓਗਜ਼ਾਲੇਟ, ਸੈਪੋਨਿਨ ਅਤੇ ਟੈਨਿਨਸ ਦੀ ਮਾਤਰਾ ਕ੍ਰਮਵਾਰ 0.65-2.08% DW, 0.23-0.63% DW, 0.19-1.04% DW ਅਤੇ 1.26- 3.30% DW ਸੀ। ਬੀਜਾਂ ਵਿੱਚ ਤੇਲ ਦੀ ਮਾਤਰਾ 10.68-27.61% DW ਦਰਜ ਕੀਤੀ ਗਈ। *ਏ. ਮੈਨੀਹੋਟ* ਅਤੇ *ਏ. ਟੈਟ੍ਰਾਫਾਇਲਸ* ਫਲੀਆਂ ਵਿੱਚ ਐਂਟੀਅਕਾਸੀਡੇਂਟਸ ਅਤੇ ਗੋਂਦ ਦੀ ਮਾਤਰਾ ਜ਼ਿਆਦਾ ਜਦੋਂਕਿ ਟੈਨਿਨਸ ਅਤੇ ਸੈਪੋਨਿਨਸ ਦੀ ਮਾਤਰਾ ਘੱਟ ਦਰਜ ਕੀਤੀ ਗਈ। *ਏ. ਮੌਸਕੇਟਸ* ਦੀਆਂ ਫ਼ਲੀਆਂ ਵਿੱਚ ਪੌਸ਼ਟਿਕ ਤੱਤਾਂ ਦੀ ਮਾਤਰਾ ਜ਼ਿਆਦਾ ਅਤੇ ਇਸਦੇ ਬੀਜਾਂ ਵਿੱਚ ਵਿਟਾਮਿਨ ਈ, ਓਲਿਕ ਐਸਿਡ, ਲਿਨਓਲਿਕ ਐਸਿਡ ਅਤੇ ਖਣਿਜਾਂ ਦੀ ਮਾਤਰਾ ਜ਼ਿਆਦਾ ਸੀ, ਜਦੋਂਕਿ *ਏ. ਟੇਟ੍ਰਾਫਾਇਲਸ* ਵਿੱਚ ਐਂਟੀਆਕਸੀਡੇਂਟਸ ਦੀ ਮਾਤਰਾ ਜ਼ਿਆਦਾ ਅਤੇ ਫਾਇਟਿਕ ਅਤੇ ਓਗਜ਼ਾਲੇਟ ਦੀ ਮਾਤਰਾ ਘੱਟ ਅਤੇ *ਏ. ਮੀਜ਼ੋਨਾਜੈਨੇਸਿਸ* ਦੇ ਬੀਜਾਂ ਵਿੱਚ ਤੇਲ ਦੀ ਮਾਤਰਾ ਜ਼ਿਆਦਾ ਦਰਜ ਕੀਤੀ ਗਈ। ਅੰਤ ਵਿੱਚ ਉਤਪਾਦਨ ਵਿਭਿੰਨਤਾ ਲਈ ਕਾਸ਼ਤ ਕੀਤੀ ਗਈ ਭਿੰਡੀ ਵਿੱਚ ਸੁਧਾਰ ਲਿਆਉਣ ਲਈ ਇਹਨਾਂ ਜੀਨੋਟਾਈਪਾਂ ਦੀ ਵਰਤੋਂ ਕੀਤੀ ਜਾ ਸਕਦੀ ਹੈ।

ਮੁੱਖ ਸ਼ਬਦ: ਭਿੰਡੀ, ਜੰਗਲੀ, ਫ਼ਲੀਆਂ, ਬੀਜ, ਪੌਸ਼ਟਿਕ ਤੱਤ, ਐਂਟੀ-ਨਿਉਟ੍ਰੀਐਂਟਸ, ਐਂਟੀ-ਆਕਸੀਡੇਂਟਸ

ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ | **ਵਿੱਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ**

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CHAPTER I

INTRODUCTION

Crop diversification is an important aspect of agriculture to cater to the undernourished of the world and the nutritionally balanced food for the burgeoning population. Vegetables form an important component of diet since they provide essential nutrients for human health. Okra (*Abelmoschus esculentus*) is an economically important vegetable crop of Malvaceae family, cultivated mainly in tropical and subtropical areas of the world (Benchasri 2012). It is addressed by different names in different parts of the globe namely lady finger in Britain, gumbo in the United States of America, guino-gombo in Spain, guibeiro in Portugal and bhindi in India. In Ethiopia its place of origin, it is called as Kenkase, Andeha, and Bamia. Its total global trade is over $5 billion (Kumar and Reddy *et al* 2015). The average productivity in India is greater than world average (12 tonnes/ha) (Chanchal *et al* 2018). In India, it was grown on 528.4 hectare area with a productivity of 11.6 MT/ha during 2016-17 (Anon 2017). Although all parts of the crop (fresh leaves, flowers, seeds and stems) are consumed by humans either as food or medicine (Sharma *et al* 2016) but immature pods are the most widely consumed part. They are consumed as vegetable, in salads, soups and stews (Gemede *et al* 2015). The okra mucilage has various health beneficial attributes *viz*. as plasma replacement or blood volume expander and has hypocholesterolemic effect (Madison 2008). Seeds contain linoleic acid rich oil (Jarret *et al* 2011) and protein, that is comparable to soybean protein in quality (Maramag 2013). Soluble fibre, present as pectins and gums, aids in lowering serum cholesterol and insoluble fibre promotes healthy intestinal tract (Hassan and Ali *et al* 2015, Georgiadisa *et al* 2011). Okra fruit also contain minerals and vitamins, which are important for human health. In addition antioxidants are also present which protect against oxidative stress and associated diseases (Gemede *et al* 2015). Okra yield and quality are affected by array of biotic stresses like viruses, fungal pathogens, insect pests and mites. Viral disease, yellow vein mosaic disease alone is responsible for 50-90% yield loss. Powdery mildew and root rot are other serious problems. Jassids, mite and fruit borer also infest okra at different growth stages and cause yield reduction of 54-66% (Lokesh 2017).

The wild relatives are used in crop improvement programmes with a view to preserve biodiversity in addition to promoting sustainable agricultural practices. They are potential sources for stress resistance and bioactives for cultivated species. Landi *et al* (2017) reported that wild relatives in Poaceae had enhanced stress tolerance in comparison to domesticated counterparts. Wild okra viz. *A. caillei*, *A. manihot* and *A. moschatus* depicted resistance against YVMD. The wild crop relatives were reported to exhibit higher phenolic content and total chlorophyll but lower nitrogen content compared to susceptible cultivars (Jakobek *et al* 2016). The wild okra accessions also exhibited resistance to insect infestation.

The crop wild relatives have since long time been used in plant breeding, contributing advantageous nutritional and agronomic traits (Pretty and Bharucha *et al* 2014, Gutiérrez *et al* 2017). For example the spelt varieties of wheat were depicted to be endowed with high antioxidant activity. As a result the spelt varieties required no pesticide use and lower nitrogen fertilization (Gawlik-Dziki *et al* 2012). Einkorn exhibited higher carotenoids than bread wheat (Shewry *et al* 2015).The flesh of non-commercial wild apple from Croatia had high polyphenol content (Jakobek *et al* 2016). The wild variants are stress tolerant as they have developed stress response mechanisms which may be attributed to higher content of antioxidants and polyphenols. Some of the wild accessions also depicted higher input use efficiency and higher productivity. Wild foods can also contribute to nutrient diversity and richness. Wild edible plants are important components of global food basket and constitute a significant part of human diet. The high nutrient and bioactive content of many wild edible plants make them valued contributors for balanced diet and play significant role in human nutrition, especially as sources of phyto-nutraceuticals. Their consumption, together with staple food crops, can provide a balanced and healthy diet to malnourished people. Substantial economic and nutrional gains have been achieved by consuming edible wild okra (Gutiérrez *et al* 2017). Loskutov *et al* (2017) reported that biochemicals like fatty acids, amino acids, and sugars exhibited wider range in wild species in comparison to the cultivated ones. The wild relatives of tomato depicted higher total soluble solids and ascorbic acid content than cultivated tomato (Kumar *et al* 2019).

The *Abelmoschus* genus contain many wild edible species such as, *A. manihot* (L.) Medik, *A. moschatus* (L.) Medik, *A. tuberculatus*, *A. manihot* (L.) Medik subsp. *tetraphyllus*, *A. mizonagenesis*, *A. angulosus*, *A. ficulneus* and *A. crinitus* (Seth *et al* 2017). These species exhibited high diversity for qualitative and quantitative characters. *A. manihot* is an annual or perennial herb, found in India, Nepal and South China. In India, it grows in Terai region and Himalayan foot hills and also in Western and Eastern Ghats. *A. moschatus* is a weedy shrub native to India and can also grow on saline wastelands (Mishra *et al* 2000). This species is distributed throughout Western and Eastern Ghats (Singh *et al* 2006). Its seeds are scented and the unripe tender pods alongwith leaves and tender shoots are consumed as vegetable. It is a rich source of phytonutrients (Pandit *et al* 2012). *A. tuberculatus*, distributed in semiarid tracts of Northern and North-Western India (Kumar *et al* 2010), is more close to *A. esculentus* than other species. *A. tetraphyllus* is subspecie of *A. manihot* (L.) Medik. It is of perennial or annual nature. It is used as clearant in jaggery industry and thus has potential commercial value. *A. angulosus* is found in high altitudes of India, Sri Lanka and Indo-China regions (Singh *et al* 2007). *A. ficulneus* and *A. crinitus* are also wild species found in Australia, Nigeria, Africa and Southern china, Nepal, India respectively. The wild *Abelmoschus* species have not been explored with respect to the biochemical composition of their green pods and mature seeds. The requisite information can be used in okra breeding programmes. The current study was undertaken with the following objectives

(i) Evaluation of immature fruits of *Abelmoschus* species genotypes for nutritional and antinutritional components

(ii) Evaluation of mature seeds of *Abelmoschus* species genotypes for oil and protein content

CHAPTER II

REVIEW OF LITERATURE

Okra improvement programmes have not received attention and impetus at global level since it is considered a minor crop (Sanjeet *et al* 2010). Biochemical analysis of germplasm can provide information for comparison of genotypes for traits that can be exploited for crop improvement for sustainable agriculture in terms of resilience, adaptation, functionality, nutrition and yield (Mahmood *et al* 2017). A crop's wild relatives harbour traits that make them hardy in the challenging environment or nutritionally better. The genetic base of modern cultivars is narrow and this poses hinderance for crop improvement efforts. Crop wild relatives (CWR) can be exploited to enhance genetic diversity in cultivated crops. The wild relatives serve as significant gene pool for improving the existing crop plants for adaptation, tolerance, yield, and nutritional quality and find application in nutraceutical, pharmaceutical, food and cosmetic industries (Wake 2012; Dubey *et al* 2016; Singh and Abhilash 2018; Whitney *et al* 2018).

2.1 Wild germplasm and crop improvement

Rahman *et al* (2017) compared the nutritional quality of wild varieties of potatoes and BARI (Bangladesh Agricultural Research Institute) released potato varieties which help to identify the superior quality of potato contributing to food and nutritional security. Various parameters viz. moisture, ash, fat, protein, crude fiber and ascorbic acid were estimated using AOAC procedures. The study revealed that moisture content in BARI released potato varieties was quite high as compared to wild varities except in Pania Alu. The maximum ash content was observed in Bish Alu (1.31%). BARI released potatoes contained high amount of ash content as compared to wild variety of yam potatoes except Bish Alu. Highest crude protein was found in BARI Alu-52 (Labadia) (2.42%) whereas wild variety namely Pania Alu contained lowest protein content (1.12%). Crude fat contents varied from 0.16% to 0.65%. The maximum fat content was found in Bish Alu (0.65%) whereas minimum value was observed in BARI Alu-55 (Red Fantasy) (0.17%) and BARI Alu-48 (0.18%). Maximum crude fiber content (1.53%) was found in Bish Alu and Pania Alu had minimum value for crude fiber (0.35%). The crude fiber content of BARI released potato varieties was in the range of 0.42-0.57%. Wild Bish Alu contained highest carbohydrate content (27.82%) as compared to released potatoes having value in the range of 21.17- 22.74%. Maximum ascorbic acid was found in 'BARI Alu-52' (Labadia) 20 mg/100 g while 'BARI Alu-60' (Vivaldi) showed minimum ascorbic acid content (12 mg/100 g). Krunic *et al* (2018) reported that introduction of recessive allele IAm (Increased Amylose), for high amylose from wild potato *(Solanum sandemanii)* into *Solanum tuberosum* resulted in enhanced amylose content upto 28-59%. They found that lack of 454 bp AFLP fragment (found in the wild species *Solanum sandemanii* (accession GCN17600) affected starch biosynthesis in potato tubers in *Solanum tuberosum*. IAm trait was transferred to tetraploid *S. tuberosum* cultivars by marker-assisted technique to increased amylose content The increase in amylose content might be due to high granule-associated phosphorylase 1 (Pho1) and decreased starch synthase (SS) activity. The level of sucrose, glucose and fructose was found to be high in tubers from the IAm lines as compared to control. Gur and Zamir (2004) also depicted that introgression of three independent yield-promoting genomic regions from the drought-tolerant green-fruited wild species *Solanum pennellii* into *Solanum lycopersicum* lead to generation of hybrids with 50% increase in yield over the leading variety under both wet and dry field conditions that received 10% of the irrigation water. Loskutov *et al* (2017) investigated seed metabolic profiles of the wild and cultivated forms of oats *(Avena* L.). Metabolic analysis was performed using gas liquid chromatoghraphy-mass spectrometry (GLC-MS). The composition and content of organic and fatty acids, amino acids, polyhydric alcohols, and sugars were analysed. Results showed that content range was narrower for the cultivated species than wild species. 33 accessions from 13 wild species and 10 accessions of cultivated oats were analysed for oil content and its quality by Leonova *et al* (2008). Oil content showed large variation among accessions of *A. canariensis* (8.6- 9.4%), *A. barbata* (6.9- 8.5%), *A. vaviloviana* (6.6- 7.6%), *A. magna* (5.2- 7.6%), *A. murphyi* (6.6- 8.8%), and *A. fatua* (6.6- 9.2%). Three species viz. *A. ludoViciana, A. hirtula,* and *A. sterilis* showed variance in the low range i.e 8.4- 8.7, 8.3- 8.7, and 6.9- 7.4% respectively. In comparison to wild oat accessions, the range in accessions of cultivated oat was from 4.1 to 6.5%. Studies also depicted that oil content had positive correlation with oleic acid and negative with FFA (Free fatty acids), TAG (Triacylglycerol), 18:2, and 18:3. Kumar *et al* (2019) studied and compared the biochemical characters of seeds and fruits of four wild relatives of tomato viz. *Solanum pimpinellifolium, Solanum chilense* (EC 513698), *Solanum lycopersicum* var. *cerasiformae* (EC 514013) and *Solanum peruvianum* (EC251790) with that of the five cultivated tomato genotypes viz Berika, BCT-115dg, Alisa Craig Aft, BCT-59 (IC 0585694) and BCT 82 (IC 0585697). The wild relatives had conspicuously higher total soluble solids (TSS) (6.59 °Brix) and ascorbic acid content (40.79 m/100 g fresh weight) than the cultivated species (4.60 °Brix, 34.09 mg/100 g fresh weight respectively). The *Solanum peruvianum* genotype had the highest vitamin C content with 38.08% more ascorbic acid content than the average of the cultivated tomato. Mean total sugar content (3.20%), reducing sugar content (2.35 %), titratable acidity (0.71 %) in fruit and total phenol content in the leaf (17.58 mg/ 100 g fresh weight) was higher in wild species, although the difference was not statistically significant. Mean soluble protein in the seed of the wild species (69.24 mg /g) was close to that of the cultivated tomato (67.73 mg/g). Top *et al* (2014) also did similar studies in fruits and reported that wild species had higher phenolic content with maximum in *S. peruvianum* accession (1619 ± 20 mg·kg^{-1} FW) followed by that in *S. pimpinellifolium* (844 ± 48 mg·kg^{-1} FW) and *S. habrochaites* (303 ± 14 mg·kg^{-1} FW) accession. Moreover, wild relatives of tomato were also used for improvement of cultivated tomato through classical breeding with regard to pest and disease resistance and abiotic stress tolerance (Ebert and Schafleitner 2015). Dubey *et al* (2015) studied the quality traits of twenty five chilli genotypes that were cultivated and consumed by the tribal people of North Eastern part of India. The genotypes had high oleoresin and capsaicin content. The total phenolic content ranged from 5.1 to 26.8 mg GAE/g DW and total carotenoids from 0.09 to 7.72 mg/g dry weight. DPPH free radical scavenging activity showed low IC50 that ranged from 0.021 to 0.041 mg/mg, low EC50 from 0.92 to 1.78 mg/mg DPPH, high ARP (Anti radical power) values (56.17– 109.52). The reducing power depicted variation from 0.92 - 4.10 ASE (Ascorbic acid equivalent)/ml. The phenolics depicted presence of gallic acid alongwith hydroxycinnamic acids. The level of catechin was maximum followed by quercetin and rutin in flavonoids. Song *et al* (2010) reported that the common cucumbers had low level of carotenoid (22- 48 µg/100 g FW) while the Xishuangbanna gourd *(Cucumis sativus var. xishuangbannanesis)* had high level of carotenoid (700 µg/100 g FW), which could be used for enhancing nutritive value of cultivated cucumber. Cuevas *et al* (2009) reported that introgression of beta-carotene genes from ‘‘Xishuangbanna gourd’’ can enhance the nutritional value of *Cucumis sativus* L. The mesocarp and endocarp samples were analyzed for beta-carotene content by reverse phase high-performance liquid chromatography (HPLC). Results were classified into two categories based on HPLC outcome. First, category included orange or high beta-carotene containing samples. Second non-orange or low beta-carotene containing samples. Saral *et al* (2014) found that wild genotypes of blueberries; *V. arctostaphylos* and *V. myrtillus* had high levels of phenolics; anthocyanins and flavonols associated with high biological activity. In *V. myrtillus* total polyphenols ranged from 11.53- 20.74 mg GAE/g dry sample, flavonoids from 1.18- 2.68 mg QE/g dry sample and anthocyanins from 3.31- 11.47 mg Cyn/g dry sample. The wild species also had high CUPRAC, FRAP and DPPH values. The Cupric Reducing Antioxidant capacity (CUPRAC), expressed as trolox equivalent antioxidant capacity varied from 0.143 to 0.297 mmol TEAC/g dry sample. The activity determined with DPPH expressed as IC50 had values from 0.229 to 1.178 mg/ml. While FRAP values expressed as $FeSO_4.7H_2O$ equivalent had a range of 130.719– 346.115 µmol Fe/g dry sample. The nutritional and antinutritional characteristics of wild fruits in Ghana; (*Gardenia erubescens*, *Sclerocarya birrea* (marula), *Diospyros mespiliformis*, and *Balanites aegyptiaca* (desert date) of dietary interest were studied by Achaglinkame *et al* (2019). These wild fruits were high in iron 0.34– 1.46 mg/100 g, zinc (0.81– 2.97 mg/100 g), vitamin A (0.84– 2.03 mg/100 g), and β-carotene (64.84–

176.89 mg/100 g). The antinutrients had values between 0.06– 1.82 mg/g. Baek *et al* (2016) studied 38 bioactive compounds, including glucosinolates, carotenoids, tocopherols, sterols, and policosanols, from nine varieties of wild Chinese cabbage *(Brassica rapa* L. *subsp. pekinensis*) to look for phytochemical variation and their relationships. The data was evaluated with principal component analysis (PCA), pearson correlation analysis, and hierarchical clustering analysis (HCA). PCA and HCA indicated two varieties; Cheonsangcheonha and Waldongcheonha, that had higher levels of glucosinolates and carotenoids. Pairwise comparisons were studied using pearson correlation coefficients. The HCA, clustered the metabolites from closely related biochemical pathways. Significant correlations were found between chlorophyll and carotenoids. Further positive correlation was observed between aliphatic glucosinolates and carotenoids. The average values of total glucosinolate and carotenoid content varied from 5.70 to 32.79 µmol/g of dry weight and 36.22 to 141.96 µmol/kg of DW respectively. The Cheonsangcheonha and Waldongcheonha varieties were found to be good candidates for breeding as they had high glucosinolate and carotenoid levels in their leaves. Feng *et al* (2012) had also identified a carotenoid rich variety in Chinese cabbage having orange coloured leaves that contain seven fold more carotenoid compounds than white cabbage. Usman and Hussan (2013) determined the nutritional and antinutritional composition of wild watermelon (*Citrullus. ecirrhosus*). Moisture varied from 3.73 ± 0.25% WW, ash content from 2.12 ± 0.08% DW ,crude protein from 26.36 ± 0.10% DW, crude lipid from 50.67 ± 0.76% DW, crude fibre from 2.17 ± 0.29% DW, carbohydrate from 18.69 ± 0.82% DW and energy value from 601. 7 ± 8.75 Kcal/ 100g DW. Evaluation of amino acids revealed that the essential amino acids except for leucine, lysine, and threonine were below the requisite values. The seed oil had 67.3% of unsaturated fatty acids (linoleic acid C:18:2 and oleic C:18:1), and 36.6% of saturated fatty acids (stearic acid C: 18.0 and palmitic acid C:16.0). High concentration of nitrate, phytate and saponin were recorded in the seed. Thus the seeds of the wild melon can be used as a potential source of nutrition.

Genetic variability for valuable fruit quality traits like high carotenoids, titrable acidity and ascorbic acid content in wild *Cucumis melo* was reported in Punjab state by Roy *et al* (2012). Thirty seven accessions of wild melon from six agro-ecological regions of the Punjab State were evaluated for total soluble solids, ascorbic acid, carotenoids and titrable acidity. Results depicted that mature fruits contained total sugars in the range of 3.0- 7.5°Brix. Ascorbic acid and tritrable acidity ranged from 3.2 to 24.4 mg/100g FW and 0.12- 1.92 % respectively. Accession WM 10 and WM 19 had significantly higher ascorbic acid content than other accessions (24.4 and 22.8 mg/100g of FW). Mean carotenoid value ranged from 76.8 to 290.5 µg/100g of FW. Nafees *et al* (2017) studied wild and cultivated pomegranate (*Punica granatum* L.) genotypes for biochemical diversity. The accessions of wild pomegranate fruits showed high morphological diversity than the cultivated genotypes. The first six principal components of PCA analysis covered 80.75 and 75.49% diversity in 53 wild and 62 cultivated pomegranate genotypes, respectively. High coefficient of variance values (10.78– 18.62%), and a high range of total soluble sugars, total phenols, titratable acidity, total soluble solids and ascorbic acid content were recorded in the studied genotypes. Ascorbic acid content showed a strong correlation with antioxidant activity, superoxide dismutase, catalase, and titratable acidity. Peroxidase depicted strong correlation with catalase, and superoxide dismutase with antioxidant activity. Based on biochemical traits, wild and cultivated pomegranates were clustered in separate groups. Holland (2009) also depicted high biochemical diversity in wild pomegranate genotypes. Wild pomegranates were high in antioxidants and phenolics and thus could be utilized in pomegranate improvement programmes. Adouni *et al* (2018) investigated the nutritional and phytochemical composition of young shoots of wild *Asparagus stipularis* from Tunisia. According to results, the young shoots are rich in valuable nutrients and dietary fiber, and their hydroalcoholic extract had high antioxidant and antiproliferative activities in the tested assays. The major phenolic compound as found by high-performance liquid chromatograph was diferuloyl glycerol. The results reinforce the traditional culinary uses of this wild plant, and that it should be included in the modern diet. Sandhi *et al* (2017) found a significant negative correlation between jassid nymphal population and tannins and total phenols and non-significant negative correlation for silica and total sugars and a positive and highly significant correlation for lignins and reducing sugars in okra. High resistance in *A. angulosus, A. moschatus and A. tetraphyllus* was related to broad leaves, high hair density, longer hair, more erect hair, high total sugars (15.21– 18.36 mg/g), low levels of reducing sugars (2.50– 3.39 mg/g), tannins (26.12– 31.48 mg/g), total phenols (1.52– 1.58 mg/g) and silica (32.66– 33.17 mg/g). *Abelmoschus manihot, A. tuberculatus,* EC0306728 and IC0506027 were found to be moderately resistant. Scholz *et al* (2016) described the variability and chemical composition of Ethiopian coffee accessions. Significant correlation among chlorogenic acids, protein and caffeine as well as between lipids and sucrose was identified. Kahweol was negatively and positively correlated with chlorogenic acids and caffeine, respectively. Greater compositional diversity was seen in Western accessions. New coffee cultivars are obtained from these accessions by increasing genetic diversity (Hamon 2015). Seth *et al* (2017) studied correlation between disease reaction and biochemical parameters of parents and hybrids at pre-flowering, flowering and post-flowering stages. The amount of total phenol and ascorbic acid and activities of peroxidase and polyphenol oxidase in okra leaf exhibited significant negative correlation with percentage disease incidence of YVMV disease over different growth stages. These might act as selection indices for identification of YVMV tolerant genotypes. Sharma *et al* (2009) reported that resistance to *H. armigera* was associated with high concentration of tannins and polyphenols and low concentration of sugars in pigeon pea. Accessions of wild relatives of pigeon pea with high concentration of tannins and polyphenols and non-glandular trichomes or low densities of glandular trichomes may be utilized in wide hybridization to create *H. armigera* resistant pigeon pea cultivars. Yilmaz *et al* (2015) reported the pomological characteristics and biochemical components in fruits of selected wild pear genotypes (*Pyrus eleagnifolia* Pall.). Fruit weight, total sugar content, total phenolics and total acidity varied from 4.71 to 27.09 g, 8.36 to 19.31 g/100 g, 42.79 to 119.14 mg GAE/100 g and 0.20 to 1.40 g/100 g respectively. The wild pears with good biochemical composition (total phenols and total sugars) could be utilized in human food. Zhang *et al* (2015) investigated differences in barley genotypes having varying response to drought stress. Under 15 % soil moisture content (SMC), when compared with control, XZ150 and XZ5 (drought tolerant) showed higher increase in soluble sugar content relative to the other three genotypes (XZ54 and XZ147 (drought-sensitive) and cv ZAU3), while XZ5 had lesser increase in soluble protein. The endogenous ABA content increased more in XZ150 and XZ5, but proline content rose least in XZ5 under 4% SMC. Lesser increase in MDA concentration was reported in XZ150 and XZ5 with higher CAT and POD activities under 4 and 15 % SMC. Drought stress at 4 % soil moisture content significantly up-regulated the levels of MnSOD in XZ150 and CAT1 and Cu/ZnSOD in XZ5. The results indicated that water deficit tolerance of wild barley XZ5 was probably related to osmo-regulation by soluble sugars and stomatal regulation of ABA. Nevo (2007) reported that durum wheat varieties were improved in protein content through crossing with wild relative, *Triticum turgidum var dicoccoides* which had high protein content. The accession FA15-3 accumulates 40% protein in presence of nitrogen. Further FA15-3 lines of wheat have been used to transfer the *Gpc-B1* gene (for high protein) into hard red spring wheat which resulted upto 3% more protein than the parallel lines. Alleles from *Oryza rufipogon* increased grain weight in a Korean wild rice cultivar (Xie *et al* 2006). Kaur *et al* (2020) reported that wild *Oryza rufipogon* accession (IRGC89224) had low expression of Phospholipase D alpha1 (OsPLDα1) enzyme which is responsible for rice bran oil rancidity. This wild accession had been deployed as the potential donor in the back-crossing programs to transfer the desired trait into elite cultivars of rice to improve shelf life of rice bran oil. Ashkani *et al* (2015) reported that wild species including, *O. minuta*, *O. autraliensis*, *O. rufipogon*, and *O. rhizomatis*, had genes for resistance against rice blast. These genes when introgressed into susceptible lines, provided protection against the disease (Sharma *et al* 2012, Wang *et al* 2014, Ashkani *et al* 2015). Ganapathy (2017) analysed two wild *(E. eoracana subsp, Africana* accession No. KH254 and KH2396) and eight domesticated cultivars of finger millet (*E. coracana subsp. coraeana*) for their proximate composition and calcium, iron, and amino acid content. Variation was observed in the protein (7.5 to 11.7%), calcium (376 to 515 mg/100 g), and iron (3.7 to 6.8 mg/100 g) content. The wild progenitor of finger millet, *E coracana subsp, africana* had significantly higher protein content. Its calcium and iron content was also significantly higher than the two domesticated cultivars (*E. coracana subsp. coraeana* accession No. KH265 and KH2333). The content of lysine and other essential amino acids was also higher. Thus the quality of fingermillet may significantly be improved by selective crossbreeding between wild and domesticated cultivars. Kaur *et al* (2019) compared fifteen cultivated (ten desi, five kabuli) and fifteen wild species of chickpea for nutritional traits, antinutritional factors and antioxidant potential. It was found that wild species *Cicer pinnatifidum* ILWC 261 had high crude protein (26.82%), lower phytic acid (8.48 mg/g) and trypsin inhibitor content (8.23 IU/g) and higher antioxidant potential (DPPH radical scavenging activity). Mezzetti (2013) reported that wild progenitor of cultivated strawberry i.e *Fragaria virginiana glauca* had significantly better nutritional quality of fruits. The fruits had significantly higher total antioxidant capacity (TAC), total phenolics, and total anthocyanins (Wang and Lewers 2007). Diamanti *et al* (2012) also confirmed the significance of wild genotyes in breeding programs for improving nutritional (i.e., TAC, total phenolics, total anthocyanins) and sensorial qualities (soluble solids, total acidity, chroma, firmness) of fruits in the prevalent cultivars.

2.2 Okra wild germplasm

Cultivated okra pods are rich source of various nutrients (Adetuyi *et al* 2011, Gemede *et al* 2014). The carbohydrate component contain cellulose, starch, sugars and mucilage (Kumar *et al* 2009, Kumar *et al* 2010). Mucilage is made up of galactose (25%), rhamnose (22%), galacturonic acid (27%) and amino acids (11%). The minerals and vitamins present in okra include- Ca, P, Fe, K, Na, I, Zn, Mn, β-carotene, riboflavin, thiamine, niacin, Vit C, Vit A, and Vit K (Kahlon *et al* 2007). Pods and seeds are rich in phenolic compounds; catechins and flavonol derivatives, while the pod skin mainly contain hydroxycinnamic and quercetin derivatives. The mature okra seed harbour oil (20% to 40%) and protein (15- 26%) (Ali *et al* 2014, Chanchal *et al* 2018). The oil mainly contain palmitic, oleic and linoleic acids (Singh *et al* 2014). The seed protein contain essential amino acids and its amino acid composition is quite similar to soybean seed protein (Hassan and Ali *et al* 2015). Among antinutrients, the phytic acid content of okra pods range from 0.06- 0.17%, oxalate content from 0.32%- 0.51%, saponins from (0.33- 0.63%) (Gemede *et al* 2014).

The *Abelmoschus* genus include about thirty eight wild species out of which about eight are edible. These include, *A. manihot* (L.) Medik, *A. moschatus* (L.) Medik, *A. tuberculatus, A. manihot* (L.) Medik subsp. *tetraphyllus, A. mizonagenesis, A. angulosus, A. crinitus* and *A. ficulneus* (Gangopadhyay *et al* 2017). *A. manihot* is found in India, Nepal and

South China. In India, it is found in Terai region and Himalayan foot hills as well as in Western and Eastern Ghats. *A. moschatus* is found in Western and Eastern Ghats of India and also in saline wastelands (Mishra *et al* 2000, Singh *et al* 2006). It tender pods,leaves and shoots are taken as vegetable. It is rich in phytonutrients (Pandit *et al* 2012). *A. tuberculatus* abounds in Northern and North-Western India (Kumar *et al* 2010) and is related to *A. esculentus* than any other species. The only difference is the presence of strigose pubescence on the stem and shorter capsule with bristly tuberculate hair. The wild variety *A. tetraphyllus* is the subspecie of *A. manihot* (L.) Medik. It grows erect to the height of 0.9– 2.0 m. It is used to clear cane juice in in jaggery industry. *A. angulosus* grows at high altitudes in India, Sri Lanka and Indo-China regions (Singh *et al* 2007). *A. crinitus* is also a wild specie found in southern china, Nepal and India. It is a perennial herb, growing to 2.0 m in height. Its root is fusiform. *A. ficulneus* is prickly annual herb, found in Australia, Africa and Nigeria. It is grown as an ornamental. Mohite and Gurav (2019) evaluated total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activities of fruit of wild *A. manihot* (L.) Medik, and *A. ficulneus* (L.) and cultivated *A. esculentus* cv. Phule Utkarsha. The maximumt total phenol content (5.86 ± 0.10 gallic acid equivalent (GAE) mg g^{-1} DW) and total flavonoid content (13.93 ± 0.19 rutin equivalent (RE) mg g^{-1} DW) were found in *A. esculentus* cv. Phule Utkarsha. *Abelmoschus ficulneus* had greater DPPH (10.65 ± 0.06%) and FRAP (33.91 ± 0.73 ascorbic acid equivalent (AAE) mg g^{-1} DW) activities. The methanolic extract had lower DPPH activity than the aqueous extract. The DPPH activity reported was similar to that in extract from leaf of *A. manihot* (Sudewi *et al* 2017) and *A. esculentus* (Tiwari *et al* 2016). The Pearson correlation analysis depicted positive correlation between fruit phenolics, flavonoids and antioxidant activity. Proximate composition of wild *Abelmoschus* was higher than the cultivated taxa with a fat content of 10.16 g/100g DW, fiber of 23.49g/100g DW, carbohydrates content 39.20g/100g DW and energy value of 273.67 Kcal/100g. *A. esculentus* cv. Phule Utkarsha had also contain higher value of copper, calcium, boron, iron, zinc, manganese, magnesium and sodium whereas minerals were low in *A.manihot*. Lalmuanthanga *et al* (2019) reported that the DPPH free radical scavenging activity, FRAP and total phenolic content were 834.60±45.84 mg/ 100 g TE, 14.00±0.69 g TE/ 100 g and 117.54mg GAE/100 gm respectively in the roots of the wild okra (*Abelmoschus moschatus*). Gul *et al* (2011) also evaluated the seeds and leaf extract of *A. moschatus* for their antioxidant and free radical scavenging activities. The antioxidant activities in terms of total phenol (9.49 to 13.84 mg GAE/g dw), flavonoids (6.0 mg QE/g dw), total antioxidant (13.30- 21.52 mg AAE/g dw) and FRAP (3.02- 6.28 mg AAE/g dw) were higher in leaf than in the seed extracts. Thus *A. moschatus*, can be used as functional foods as well as pharmaceutics. Pravin *et al* (2018) studied 60 accessions of eleven

Abelmoschus species to estimate genetic diversity and species relationships. Among the studied species, high genetic diversity (H) was observed in *A. manihot* subsp. *tetraphyllus* var. *tetraphyllus*, whereas lowest genetic diversity was observed in *A. manihot* subsp. *tetraphyllus* var. *pungens*. Among the studied species highest (0.9095) similarity index was observed between *A. esculentus* and *A. caillei* whereas *A. palianus* and *A. crinitus* exhibited lowest (0.5149) similarity index. Moreover, level of genetic diversity was found to be low (Percentage of polymorphic loci (PPL) = 13.53% to 13.86%) in cultivated species populations than wild species populations (PPL = 4.29% to 62.05%). The UPGMA (Unweighted pair group method with arithmetic mean) based cluster analysis clearly distinguishes the *Abelmoschu*s species into three major clusters – I, II and III. Gangopadhyay *et al* (2017) evaluated and characterized sixty eight (68) accessions including four wild *Abelmoschus* species (*A caillei (A. Chev.) Stevels, A manihot* (L.) Medik*, A moschatus* (L.) Medik. and *A tuberculatus* and eight okra varieties for phenological characters including biotic stresses under natural condition. The wild species included eighteen accessions (16 exotic and 2 indigenous) of *A. caillei*, twenty nine of *A. manihot*, sixteen of *A. moschatus* and five of *A. tuberculatus*. The Shannon diversity index was high (≥0.4) for the wild *Abelmoschus* species for qualitative characters viz. stem colour intensity, leaf shape, epicalyx shape, 13 quantitative characters and 3 biotic stress parameters. *A. caillei* and *A. tuberculatus* had maximum and minimum diversity for qualitative characters, respectively. Resistance to YVMD was found in accessions of three wild species viz. *A. caillei*, *A. manihot* and *A. moschatus* while resistance to shoot and fruit borer and leaf hopper was found in accessions of all the wild species under study. The resistant accessions can be used for introgressing resistance trait through crossing into cultivated species. Waris *et al* (2016) studied and compared the flavonoid free radical scavenging activity of leaf extracts of *A manihot* (L.) Medik, with the quercitin free radical scavenging activity. The leaf tissue was extracted with different solvents viz. n-hexane, ethyl acetate and ethanol. The IC50 values for n-hexane extract was 35.83 µg/mL, for ethyl acetate extract it was 19.50 µg/mL and for ethanol extract a value of 12.36 µg/mL. Taroreh and Raharjo (2016) reported that the total phenolic content of 297.43±0.48 mg GAE/g and flavonoids content of 117.31±0.38 mg quercetin equivalent/g were obtained in the leaf extract of *A. manihot*. Anggi and Adikusuma (2019) found that the total antioxidant activity of leaf extract of *A Manihot* from Palu of Central Sulawesi was 3.45 µg/ml and show toxicity towards breast cancer 4T1 cell lines, and can act as natural antioxidant and for preparation of anti-breast cancer drug. Nair and Fahsa (2013) studied mucilage from five species of *Abelmoschus*; two cultivated (*A. esculentus, A.caillei*) and three wild (*A. manihot, A.angulosus and A.moschatus*). The various properties viz. physical, physiochemical and phytochemical, of the mucilage were analyzed using standard procedures.

The mucilage yield was highest at 4 DAF (Day after flowering) in all the samples, decreasing subsequently (8DAF, 12DAF, 16 DAF). The highest value was found in *A. esculentus* (0.571 g/g at 4 DAF), followed by that in *A. manihot* (0.382 g/g at 4 DAF) and *A. moschatus* (0.357 g/g at 4 DAF). *A caillei*, had lower values (0.229 g/g at 4DAF). *Abelmoschus angulosus* had the lowest mucilage yield. The moisture content was relatively high in *A.esculentus*, and low in *A.moschatus*. Swelling index values were higher in distilled water compared to HCl (0.1N) and phosphate buffer. The yield of water soluble extracts for all the samples, were higher compared to the alcohol extracts. The values were highest for *A. moschatus* (96%) followed by *A. caillei* (94%). The water-soluble ash was highest in *A. esculentus* (3.5%) followed by *A. manihot* (3.1%) and lowest was for *A. moschatus* (2.5%). The acid-insoluble ash values were comparatively very low for all the species. All samples had pH values above 5. Near-neutral values were in the order; *A. angulosus* > *A. esculentus* >*A. moschatus*>*A. caillei*> *A. manihot*. Regarding the suspending property, *A. moschatus* was at par with that of *A. esculentus* mucilage and can be beneficially exploited for mucilage isolation. Adetuyi and Dada (2014) studied the composition of okra (*Abelmoschus esculentus*), water leaf (*Talinum triangulare*) and Jews mallow (*Corchorus olitorius*) mucilage. The mucilage from three sources had protein content of 20.30, 54.30, 44.80%; fiber content of 2.0, 3.50, 8.25%; fat content of 31.0, 29.0, 33.8%; ash content of 5.0, 7.80, 5.0% and carbohydrate content of 41.70, 5.40, 8.15 % respectively. Qiu and Song (2012) estimated total flavonoid activity (TFA) from *A. manihot* flowers ethanol extract (70%). The superoxide anions and hydrogen radical scavenging were related to the total flavonoid concentrations with the IC50 values of 63.90 ±2.21 µg/mL and 266.88±28.32 µg/mL, respectively. The IC50 values of standard ascorbic acid were 436.52 ±14.36 µg/mL and 439.58±21.41 µg/mL, respectively. The DPPH radical scavenging activity increased with increasing of TFA concentrations and the highest inhibition was 94.63 ±3.01% at 50 µg/mL, as compared to that of ascorbic acid 34.94 ±1.50%. The extract also had good reducing power. The *A. manihot* flower extract had significant potential for use as health supplements and nutraceuticals. Jarret *et al* (2011) evaluated 1100 genebank accessions of okra (*Abelmoschus esculentus*) and 540 additional accessions that included some cultivated and wild species—*A. caillei, A. crinitis, A. esculentus, A. ficulneus, A. manihot, A. moschatus* and *A. tuberculatus* for seed oil content using time domain NMR (TD-NMR). Oil content in seeds of *A. caillei, A. esculentus, A. ficulneus, A. manihot, A. moschatus and A. tuberculatus* ranged from 2.51- 13.61%, 12.36- 21.56%, 6.62- 16.7%, 16.1- 22.0%, 10.3- 19.8% and 10.8- 23.2%, respectively. Accession PI639680 of *A. tuberculatus* had the highest value (~23%). Accessions of *A. esculentus* that had high seed oil content included PI nos. PI274350 (21.5%), PI538082 (20.9%) and PI538097 (20.9%). Three accessions of *A. manihot* with the highest seed oil content were PI nos. PI639673 (20.4%), PI639674 (20.9%) and PI639675 (21.9%), all belonging to var. *tetraphyllus*. Ninety-eight accessions from six species were also evaluated for fatty acid composition. Linoleic acid content ranged from 23.6- 50.65% in *A. esculentus*. The mean linoleic acid concentrations was highest in *A. tuberculatus* and *A. ficulneus*. Palmitic acid contents were significantly higher in *A. esculentus* (range of 10.3- 36.35%). The levels were also high in *A. caillei* (mean = ~30%). Levels of oleic acid were highest in *A. manihot*, *A. manihot* var. *tetraphyllus* and *A. moschatus*. Knani *et al* (2019) determined phenolic, chlorophyll, carotenoid and mineral content (Ca, Mg, Na, K, Fe, Zn, P) in the seed oil of the three Iraqi cultivars of okra (*Abelmoschus esculentus* L. The results showed that the husayniyah seed oil contained the highest pheolic, chlorophyll and caroteniod content with value of 34.73mg Gallic acid/100g oil, 2.295mg/kg oil and 2.813mg/kg oil respectively while Batera seed oil contained maximum value of vitamin E (3861.347 ppm). But with respect to minerals, Petra seed oil had greater content of Mg (321 ppm) while phosphorus was (382.799ppm) maximum in husayniyah. Pascal *et al* (2018) also studied mineral composition in fresh immature fruits of four varieties of *Abelmoschus caillei* (V55, V57, V58 and V60) and one variety of Abelmoschus moschatus (V61). Results depicted that Iron, copper and Zinc content varied from 28- 36 mg/ kg, 8- 12 mg/ kg and 32- 58 mg/ kg DW respectively.

CHAPTER III

MATERIALS AND METHODS

The present study was carried out to evaluate wild *Abelmoschus* species for diversity of biochemical traits in immature pods and mature seeds. The evaluation aims at searching for genotypes with better quality traits, which may be used in okra breeding programmes. The following materials and methods were used in the study.

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3.2.4.2 Carotenoids (fruits and seeds)

3.2.4.3 Total phenols

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3.2.4.5 Flavanols

3.2.4.6 Tocopherols

3.2.4.7 Ascorbic acid

3.2.4.8 Total antioxidant activity

3.2.4.8.1 DPPH activity

3.2.4.8.2 FRAP activity

3.3 Statistical analysis

3.1 Materials

The material consisted of immature fruits and seeds of wild *Abelmoschus* species listed in Table 3.1.

Table 3.1: *Abelmoschus* species used in the study

Name of *Abelmoschus* species	Accession No. / Variety
Abelmoschus moschatus	IC 141056
	IC 140986
	IC 140985
	IC 470737
Abelmoschus mizonagenesis	_
Abelmoschus tetraphyllus	_
Abelmoschus angulosus var.grandiflorus	IC 203833
Abelmoschus tuberculatus	_
Abelmoschus manihot	_
	IC 90339
Abelmoschus esculentus	Punjab Padmini

3.1.1 Collection of sample

The material was collected from fields of Department of Vegetable Science, Punjab Agricultural University, Ludhiana. The immature pods were taken after 45 days of sowing and stored in deep freeze at -20°C till further analysis. The seeds were collected from mature pods.

3.2 Biochemical analysis

Immature pods and mature seeds were evaluated for the following biochemical parameters.

3.2.1 Moisture (AOAC 1999)

Procedure

The empty dish was dried for 1 h at 70°C in oven, kept in dessicator, cooled and weighed. Placed 5 g of sample in the dish and kept the dish in oven at 105°C for 3 h for drying. After that dish was placed in desiccator, cooled and reweighed. Moisture content was calculated as:

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

where:

W_1 = sample weight (g) before drying

W_2 = sample weight (g) after drying

3.2.2 Nutritional parameters

3.2.2.1 Total carbohydrates (Hedge and Hofreiter 1962)

Principle: Carbohydrates are hydrolyzed by HCl into simple sugars. The acid dehydrates sugars to furfural and hydroxy-methyl furfural, which react with anthrone to form green coloured product whose intensity is read at 630 nm.

Reagents

i. 2.5 N HCl: 9.12 ml of HCl was dissolved in 90.88 ml of DDW.

ii. Anthrone reagent: Freshly prepared by dissolving 200 mg anthrone in 95% ice cold sulphuric acid before use.

iii. Glucoe standard (100 μg/ml): 10 mg glucose was dissolved in 80 ml of DDW and finally the volume was made to 100 ml with DDW.

Procedure

Weighed 0.2 g of sample and homogenized in 2 ml of 2.5 N HCl. Transferred the contents to 2 ml micro-centrifuge tubes and placed in boiling water bath for 3 h for hydrolysis. Cooled the sample to room temperature and added sodium carbonate until effervescence ceased and centrifuged at 8,000 rpm for 10 min. Took 20 μl of supernatant and added 980 μl DDW. This was followed by addition of 4 ml of anthrone reagent. The tubes were placed in boiling water bath for 8 min and then cooled rapidly and absorbance was read at 630 nm. A standard curve for glucose in the range of 10- 100 μg was also run. The total carbohydrate content was expressed as g/100 g dry weight (DW) tissue.

3.2.2.2 Total soluble sugars (Dubois *et al* 1956)

Extraction

Reagents

i. 80% ethanol: Took 80 ml of ethanol and made the volume to 100 ml with DDW.

ii. 70% ethanol: Took 70 ml of ethanol and made the volume to 100 ml with DDW.

iii. Saturated lead acetate solution: Dissolved 17.5g of lead acetate in 50ml of DDW.

iv. Sodium oxalate.

Procedure

Tissue (0.2 g) was refluxed in 20 ml of 80% aqueous ethanol for 1h. Centrifuged the contents at 8000 rpm for 15 min. The residue was extracted twice with 70% aqueous ethanol.

The supernatant was collected and pooled. The final pooled extract was evaporated to dryness at 50^0 C in flash evaporator under vacuum. 1 ml of saturated lead acetate solution was added to extract and made the volume to 10ml with DDW. Kept the solution overnight for proteins to precipitate and filtered the extract. Then added a pinch of sodium oxalate (to remove excess lead ions) and kept the solution overnight. Filtered the extract through whatmann No.1 filter paper. The clear extract obtained was used for estimation of total soluble sugars and reducing sugars.

Estimation

Principle: Sugars (pentoses and hexoses) form furfurals and hydroxyl-methyl furfurals in the presence of concentrated sulphuric acid. The products react with phenol to give orange colored complex, which is read at 490nm.

Reagents

i. Concentrated H_2SO_4.
ii. Phenol (5%): 5 g phenol was dissolved in 100 ml of distilled water and kept in amber colored bottle.
iii. Glucose standard (100 µg/ml): Prepared by dissolving10 mg of glucose in 80 ml of DDW and finally made the volume to 100 ml with DDW.

Procedure

Took 50 µl of extract and added 450 µl of DDW followed by 0.5 ml of 5% phenol. Cyclomixed the contents and added 2.5 ml of concentrated H_2SO_4 in the center of tubes to ensure proper mixing and raising the temperature of the reaction mixture to about 70˚C. This would cause optimal color development. The tubes were kept at room temperature for 10 min and thereafter absorbance was measured at 490 nm against blank. Standard was run taking glucose in the range of 10-60 µg. Total soluble sugar content was expressed as g/100 g DW tissue.

3.2.2.3 Reducing sugars (Miller 1972)

Principle: 3,5-Dinitrosalicylic acid reacts with reducing sugars to form 3-amino-5-nitrosalicylic acid, which absorbs light strongly at 540 nm, with simultaneous oxidation of aldehyde and ketone functional groups of reducing sugars under alkaline conditions.

Reagents

i. Dinitrosalicylic acid (DNSA) reagent: Dissolved 1 g of DNSA, 0.2 g of crystalline phenol, 0.05 g of sodium sulphite in 100 ml of 1% NaOH solution. Sodium sulphite should be added at the time of use because it deteriorates the reagent. Reagent was stored at 4°C.
ii. Potassium sodium tartrate (40%): 40 g of potassium sodium tartrate was dissolved in 100 ml of DDW.

iii. Standard glucose (100 μg/ml): Prepared by dissolving 10 mg of glucose in 80 ml of DDW and finally made the volume to 100 ml with DDW.

Procedure

To 0.2 ml of extract, added 2.8 ml of DDW followed by 3 ml of DNSA reagent. Placed the tubes in boiling water bath for 10 min until the color changes from yellow to red. Then added 1 ml of 40% potassium sodium tartrate solution. Cooled the contents and took the absorbance at 510 nm. Standard glucose was run simultaneously in the range of 10-60 µg. The content of reducing sugars was expressed as g/100 g DW tissue.

3.2.2.4 Non-reducing sugars

The difference between the total soluble sugars and the reducing sugars will give non reducing sugar content.

3.2.2.5 Mucilage (Farooq *et al* 2013)

Reagent

i. Acetone.

Procedure

Took 25 g of chopped tissue in 50 ml of DDW in a beaker. The contents were placed at 60 °C for 6 h with continuous stirring for complete extraction of mucilage. The sample was filtered through muslin cloth and cooled to room temperature. Added 150 ml of acetone. The precipitates were collected and rewashed with acetone and then dried at 45° C for 4 h. On drying the sample became hard and brownish in color. Weighed the mucilage obtained and expressed it as g/100 g DW tissue.

3.2.2.6 Crude protein (Pearson 1981)

Principle: The nitrogen in the sample is converted to ammonium sulphate on treatment with concentrated sulphuric acid. Distillation with excess alkali liberates ammonia that gets trapped in 2% boric acid and is estimated by titration with hydrochloric acid.

Reagents

i. Concentrated sulphuric acid.
ii. Catalyst mixture: potassium sulphate: copper sulphate (3:1).
iii. 40% Sodium hydroxide: 40 gm of sodium hydroxide was dissolved in 100 ml of DDW.
iv. 0.01N HCl: Took 0.036 ml of HCl and made the volume to 100 ml with DDW.
v. Methyl red-bromocresol green indicator: Dissolved 0.3 g of bromocresol green and 0.2 g of methyl red in 400 ml of 90% ethanol.

Procedure

Digestion

Transferred 0.1 g of sample to digestion flask. Added 10 ml of sulphuric acid and 2 g of catalyst mixture to it. Digested for 2 h. Initially the temperature was kept low and then increased to 390°C until clear solution was obtained. After cooling the sample was transferred to 50 ml volumetric flask and volume was made to 50 ml with DDW.

Distillation

Pipetted out 10 ml of the diluted sample into the distillation unit and added 20 ml of 40% sodium hydroxide solution and started distillation. On distillation, the liberated ammonia gas gets trapped in 10 ml of 2% boric acid solution having 4 drops of methyl red-bromocresol green mixed indicators, contained in 100 ml conical flask. The low pH of solution converted boric acid into borate and ammonia gas into ammonium ions. After 10 min the distillation was stopped.

Titration

The boric acid solution containing trapped ammonia was then titrated against 0.01 N hydrochloric acid till bluish green color changes to pink. The volume of 0.01 N HCl used was applied to calculate the content of nitrogen (%). The nitrogen content (%) was multiplied with a factor of 6.25 to get crude protein content (%).

3.2.2.7 Seed soluble proteins (Lowry *et al* 1951)

Extraction

Reagents

i. 0.1N NaOH: 0.4 g NaOH was dissolved in 50 ml of DDW and final volume was made to 100 ml with DDW.
ii. 20% TCA**:** 20 g of TCA was dissolved in 100 ml of DDW.

Procedure

Extracted 0.2 g of seed flour with 5 ml of 0.1 N NaOH and centrifuged the extract at 4° C for 15 min at 6000 rpm. The extraction was repeated and supernatants pooled. Pipetted out 2 ml of supernatant and added 2 ml of chilled 20% TCA, mixed and kept for 1 h at 4^0 C. The contents were centrifuged at 4° C for 15 min at 6000 rpm. The supernatant was discarded and the residue was dissolved in 5 ml of 0.1 N NaOH and used for protein estimation.

Estimation

Principle: The peptide bonds (–CO-NH-) of a polypeptide chain react with alkaline copper sulphate to form blue coloured complex. In addition, the tryptophan and tyrosine residues of the protein also reduce the phosphotungstate and phosphomolybdate components of Folin Ciocalteau reagent to give bluish product which increases the sensitivity of the method.

Reagents

i. Reagent A: 2 g of sodium carbonate was dissolved in 100 ml of 0.1N NaOH.
ii. Reagent B: 0.5 g of copper sulphate ($CuSO_4$) and 1 g of sodium potassium tartarate were dissolved in 100 ml of DDW.

iii. Reagent C: Reagent A and reagent B were mixed in the ratio of 50:1 prior to use.
iv. Reagent D: Folin-Ciocalteau reagent and DDW were mixed in the ratio of 1:1 before use.
v. BSA standard: 10 mg of bovine serum albumin (BSA) was dissolved in minimum quantity of DDW and finally volume was made to 100ml with DDW.

Procedure

Took 0.1 ml of extract and added 0.9 ml of DDW and 5 ml of Reagent C. The contents were vortexed. After keeping tubes for 10 min at room temperature, added 0.5 ml of reagent D. Mixed and kept the tubes for 30 min at room temperature. The blue color developed was read at 520 nm against reagent blank**.** Standard was run in the range of 20-200 µg. Protein content was expressed as g/100 g DW tissue.

3.2.2.8 Total free amino acids (Blackburn 1968)

Principle: Ninhydrin is reduced when heated with aqueous solution of free amino acids. The amino acid is oxidized to an aldehyde with one carbon less, with the release of carbon dioxide. The reduced ninhydrin condenses with ammonia and ninhydrin to form a blue coloured complex.

Reagents

i. 0.5 M Citrate buffer (pH 5.5): Solution A: 0.5 M citric acid was prepared by dissolving 10.51 g of citric acid in 80 ml of DDW and finally made the volume to 100 ml with DDW. Solution B: 0.5 M sodium citrate was prepared by dissolving 14.7 g of sodium citrate in 80 ml of DDW and finally made the volume to 100 ml with DDW. For preparing 0.5 M Citrate buffer, 14.85 ml of solution A was mixed with 35.5 ml of solution B. The pH of resulting solution was adjusted to 5.5. The final volume was made to 100 ml with DDW.
ii. 1% Ninhydrin solution: 1g of ninhydrin was dissolved in 100 ml of 0.5 M Citrate buffer.
iii. Pure glycerol.
iv. Ninhydrin Reagent- Ninhydrin solution: glycerol: citrate buffer: 5:12:2
v. Tris buffer (25mM, pH 8.3): 0.3 g of tris was dissolved in 80 ml of DDW and finally volume was made to 100 ml with DDW..
vi. Standard glycine 1µg/ml: 0.1 g of glycine was dissolved in small amount of water and final volume made to 100 ml with distilled water.

Procedure

Homogenised 0.2 g of tissue in 2 ml of 25 mM tris buffer (pH 8.3). Centrifuged the homogenate at 4^0 C at 8,000 rpm for 20 min. To the supernatant, added 200 µl of saturated lead acetate solution and kept overnight at room temperature. Again centrifuged the contents and took 200 µl supernatant and added 800 µl of citrate buffer. Vortexed the contents and added 4 ml of ninhydrin reagent. Kept the tubes in boiling water bath for 20 min. After cooling, absorbance was read at 570 nm. Standard was run in the range of 10-50μg. Amino acid content was expressed as g/100 g DW tissue.

3.2.2.9 Seed oil (Folch *et al* 1957) and fatty acid composition (Appleqvist 1968)

Seed oil

Principle: The seed powder is extracted in chloroform: methanol mixture (2:1) for 24 h. As the solvent gather traces of sugars, amino acids and proteins along with lipids, their removal is carried out by Folch washings given with 0.9% NaCl.

Reagents

i. Chloroform:methanol :: (2:1)

ii. 0.9% Saline solution: 0.9 g of NaCl was dissolved in 100 ml of DDW.

Procedure

Ground dried 0.5 g seed in pestle motor using pinch of anhydrous sodium sulphate and transferred contents to 100 ml conical flask containing 20 ml of chloroform: methanol mixture (2:1) for extraction for 24 h. After 24 h, filtered the contents through G-3 sintered glass funnel. Transferred the filterate into separatory funnel and added $1/5^{th}$ of extract volume of 0.9% NaCl solution. The mixture was shaken gently for 30 min and kept overnight for separation of layers. Collected the lower layer. Washed the upper layer twice with 5 ml chloroform and collected the lower layer and pooled the collected layers. Evaporated the pooled sample on flash evaporator. Added chloroform to residue and made the volume to 25 ml with chloroform. Took 5 ml of final extract into a preweighed vial and dried in oven at 50° C till contant weight is obtained. Then calculated the oil content (%).

Fatty acid composition

Principle: The fatty acids are converted as fatty acid methyl esters which volatilize at 200^0C and get separated on gas liquid chromatograph.

Reagents

i. Petroleum ether (40°-60^o C).

ii. 0.02 M Sodium ethoxide: 0.136 g of sodium hydroxide was dissolved 50 ml of absolute alcohol and final volume was made to 100 ml with absolute alcohol.

iii. NaCl (8%): 8g of NaCl was dissolved in 100 ml of DDW.

Procedure

Weighed 0.05 g of dry seed powder and added 1.5 ml of petroleum ether into it. Vortexed the tubes and kept for 30 min. Added 1.5 ml sodium ethoxide (0.02 M). Vortexed the tubes and kept at room temperature for 30 min. Added 1.5 ml of 8% NaCl solution and vortexed again. After 30 min two layers were formed. Transferred the upper petroleum ether layer into GC vials of 1.5 ml capacity and 1μl of it was infused into GC on Agilent technologies Gas Chromatograph Model 7820A series outfitted with flame ionization detector and fitted with CP-Sil 88 (25 m x 0.25 mm x 0.20 mm) FAME column.

Optimum conditions of GLC

Oven temperature	:	180-200^o C
Detector temperature	:	240^o C
Injector temperature	:	230-240^o C
Air flow	:	300 ml/min.
Hydrogen flow	:	30 ml/min.
Nitrogen flow	:	60 ml/min.
Column solid support	:	Polyimide coating fused silica
Column stationary phase	:	(50%-Cyanopropyl)- methylpolysiloxane

EZ Chrome elite software was used to calculate the relative concentration of fatty acids

3.2.2.10 Crude fiber (AOAC 1984)

Principle: The dried sample is digested first with a weak acid solution, then with a weak base solution. The organic residue is collected in a filter crucible and weighed. The loss of weight on ignition in muffle furnace gives crude fiber.

Reagents

i. Hydrochloric acid (1%): Mixed 1 ml of HCl and 99ml of DDW.

ii. Sulphuric acid (0.255 N): Added 2.5 ml of sulphuric acid to 97.5 ml of DDW.

iii. Sodium hydroxide (0.313 N): Dissolved 1.25 g of NaOH in 80 ml of DDW and finally made the volume to100 ml with DDW.

Procedure

Took 25 g of fresh okra pods and transferred to 500 ml beaker. Added 200 ml of 0.255 N sulphuric acid and digested the sample at 100° C for 30 min. The contents were filtered through Buchner funnel, and washed with distilled water. The residue was digested using 200 ml of 0.313 N sodium hydroxide for 30 min. The residue was filtered and washed with 1% HCl to neutralize sodium hydroxide and rinsed with DDW. After washing, it was dried in hot air oven for 1 h and ashed in muffle furnace at 500^0 C for 8 h, cooled and reweighed.

$$\text{Crude fiber (\%)} = \frac{W_1 - W_2}{W_S} \times 100$$

Where, W_S = Weight of sample

W_1 =Weight of crucible+ sample

W_2 =Weight of crucible+ ashed sample

3.2.2.11 Ash (AOAC 1990)

Principle: Sample is ignited at 600^{0}C to burn off all organic matter. The inorganic material which does not volatilize at that temperature is called ash.

Procedure

Took 5 g of tissue in preweighed crucible and kept in muffle furnace at 600° C for 6 h. White ash was obtained and the crucible was immediately shifted to dessicator and weighed after cooling. Ash percentage was calculated as follows:

$$\% \text{ ASH} = \frac{\text{Weight of crucible and ash - weight of crucible}}{\text{Weight of crucible and sample - weight of crucible}} \times 100$$

3.2.2.12 Mineral composition

Principle: When sample is exposed to plasma energy, the atoms of component elements get excited and when these return to low energy position, the emitted rays that correspond to the photon wavelength are measured.

Reagents

i. Perchloric acid (70%): Mixed 70 ml of perchloric acid and 30 ml of DDW.
ii. Nitric acid (70%): Mixed 70 ml of nitric acid and 30 ml of DDW.
iii. Nitric: perchloric acid solution: Nitric acid and perchloric acid were mixed in the ratio of 2:1.
iv. Standard solution of iron, zinc, copper, calcium, magnesium, potassium, phosphorus and manganese

Procedure

Digested 0.5 g of seed flour with 10 ml of Nitric: Perchloric acid solution till a clear solution was obtained. The solution was cooled and final volume was made to 25 ml with DDW and filtered through Whatman No.1 filter paper**.** For the estimation of minerals ICP-OES (Inductively coupled plasma optical emission spectroscopy) method is used which involved the production of excited atoms and ions that emitted electromagnetic radiation at wavelengths characteristics of particular element. Instrument optimization, calibration and mineral analysis were carried out using WinLab 32 Software.The standard of each mineral were used to prepare standard curve for calibration. The mineral content was expressed in ppm**.**

3.2.2.13 Iodine (Mahesh *et al* 1988)

Principle: The estimation is based on Ce^{4+} - As^{3+} redox reaction evaluated by kinetic assay. The reduction of Ce^{4+} to Ce^{3+} is coupled with the oxidation of As^{3+} to As^{5+}. The reduction of Ce^{4+} is monitored by decrease in absorbance at 370 nm for 1 min. The rate of disappearance of yellow colour is analysed as a measure of iodine content.

Reagents

i. Potassium hydroxide (6 M): 33.67g of KOH was dissolved in 60 ml of DDW and final volume was made to 100 ml with DDW.

ii. Zinc sulphate (0.52 M): Dissolved 8.30 g of zinc sulphate in 80 ml of DDW and final volume was made to 100 ml with DDW.

iii. Sulphuric acid: Hydrochloric acid solution: (0.7 N H_2SO_4 and 0.06 N HCl): Combined reagent of H_2SO_4 and HCl was prepared by mixing 9.8 ml of conc. H_2SO_4 and 250 ml of water, cooled to room temperature and then 2.7 ml of HCl was added to it and final volume was made to 500 ml with DDW.

iv. Arsenic Reagent (0.03 M): 0.593 g of Arsenic trioxde and 0.6 g of KOH were mixed in 30 ml of DDW and then 0.1 ml of conc. HCl was added and final volume was made to 100 ml with DDW.

v. Cerrate Reagent (0.05 M): Dissolved 0.316 g of ammonium ceric sulphate in 15 ml of DDW and added 40 ml of conc. nitric acid dropwise followed by addition of 5 ml of conc. H_2SO_4 and final volume was made to 100 ml with DDW.

vi. Standard Iodine solution (0.1 mg/ml): Solution A: 130.8 g of anhydrous potassium iodide was dissolved in few ml of DDW and final volume was made to 1 liter with DDW.

Solution B: Diluted 10 ml of solution A to 1 liter with DDW.

Solution C (100 µg/ml working solution): Diluted 10 ml of solution B to 100 ml with DDW.

Ashing

Dried and finely ground (1 g) of seed sample was transferred to clean test tubes. Added 0.1 ml of 6 M KOH and kept for 24 hrs for drying in oven at 95± 1°C. The samples were incinerated in muffle furnace for 6 h at 600° C with renewal of air for 15 sec after every 15 min. The samples were transferred to dessicator and cooed to room temperature. If, ashing is incomplete then added 1 ml of 10 ml diluted zinc sulphate solution and continued drying and ashing as above. To the ashed sample, added 0.2 ml of DDW, mixed and stirred thoroughly and volume was made to 5 ml with DDW. The sample was centrifuged at 3500 rpm for 30 min and supernatant was kept for analysis of total iodine.

Procedure

Added 0.5 ml of DDW, 0.5 ml of H_2SO_4: HCl solution, 0.5 ml of cerate reagent and 0.5 ml of arsenic reagent to cuvette. Added immediately 0.5 ml of catalyst iodine (blank/standard/sample) at the end to initiate the reaction. The decrease in the absorbance was recorded for 1 min at 370 nm. The disappearance of yellow colour in Ce^{4+} to As^{3+} reaction indicated the measure of iodine content. Standard curve was prepared using 0, 3, 4, 5, 6, 7 and 8 µg/ml of iodine respectively.

$$\text{Iodine (mg)} = \frac{As - Ab}{m} \times d$$

Where As = change in A/min in sample

Ab = change in A/min in blank

m = slope of standard curve

d = dilution in ml

3.2.3 Antinutritional parameters

3.2.3.1 Phytate (Fruhbeck *et al* 1995)

Principle: Phytate estimation is based on reaction between the ferric ion (Fe^{3+}) and the sulfosalicylic acid (wade reagent) with the formation of complex that is spectrophotometrically monitored at 500 nm. The presence of phytate decreases the intensity of pink color.

Reagents

i. 2.4% Hydrochloric acid: Mixed 2.4 ml of HCl and 97.6 ml of DDW.

ii. 0.3% Sulphosalicylic acid: Dissolved 0.3 g of sulphosalicylic acid in 100 ml of DDW.

iii. Wade reagent: Dissolved 0.03 g $FeCl_3.6\ H_2O$ in 100 ml of 0.3% sulfosalicylic acid. Stored the solution in dark to avoid oxidation.

iv. Standard phytate (100µg/ml): Prepared by dissolving 4 mg of sodium phytate in 100 ml of DDW. The stock solution was diluted to get concentration of 100µg/ml.

Procedure

Homogenized 0.2 g of tissue in 2 ml of 2.4% HCl solution. Homogenates were kept on shaker for 2 h at room temperature and then centrifuged at 10,000 rpm for 20 min. To 0.2 ml of supernatant added 2.8 ml DDW followed by addition of 1 ml wade reagent and incubated at room temperature for 10 min in dark. Absorbance was measured at 500 nm against reagent blank. Standard curve was run in the range of 10-100µg/ml. The content of phytate was expressed as g/100 g DW tissue

3.2.3.2 Tannins (Price *et al* 1978)

Reagents

i. Hydrochloric acid (1%) in methanol: Mixed 1 ml of HCl and 99 ml of methanol.

ii. Vanillin reagent (1% vanillin in 8% methanolic HCl): 1 g of vanillin was dissolved in 100 ml of 8% methanolic HCl (Mixed 8 ml of HCl and 92 ml of methanol).

iii. Standard catechin (1mg/ml): Dissolved 0.1 g of catechin in minimum DDW and made the volume to 100 ml with DDW.

Procedure

Homogenised 0.2 g of tissue in 2 ml of 1% methanolic HCl. The contents were vortexed for 20 min at room temperature and centrifuged at 10,000 rpm for 10 min. Pipetted out 0.5 ml of supernatant into test tube and added 2.5 ml of vanillin reagent. Incubated the contents at 30° C for 20 min and measured the absorbance at 500 nm. The standard was run in the range of 10-100µg/ml. The content of tannins was expressed as mg/100 g DW tissue.

3.2.3.3 Oxalate (Day and Underwood 1986)

Reagents

i. H_2SO_4 (3 M): Added 16.3 ml H_2SO_4 to 83.7 ml DDW.

ii. $KMnO_4$ (0.05M): 0.790 g $KMnO_4$ was dissolved in 80 ml of DDW and final volume was made to 100 ml with DDW.

Procedure

Took 5 g of sample in 100 ml conical flask. Added 75 ml of 3 M H_2SO_4 in the flask and stirred the contents for 1 h on magnetic stirrer. The contents were filtered through Whatman No. 1 filter paper. 25 ml of filterate was taken and titrated while hot against 0.05 M $KMnO_4$ solution until a faint pink color was obtained which was stable for 30 sec. The oxalate content (g/100 g DW) was calculated by taking 0.05 M $KMnO_4$ equivalent to 2.2 mg oxalate.

3.2.3.4 Saponin (Fenwick and Oakenfull 1983)

Reagents

i. Acetone.

ii. Methanol.

iii. Ethyl acetate.

iv. Reagent A- 0.5ml of anisaldehyde was dissolved in 99.5ml of ethyl acetate.

v. Sulphuric acid.

Procedure

Homogenised 500 mg of tissue in 5 ml of acetone and kept overnight at room temperature followed by removal of acetone. The extraction was repeated with 5 ml of methanol for 24 h. The methanol extract was filtered and volume was made to 12.5 ml with methanol. Pipetted out 1 ml extract into test tube and evaporated methanol by placing in boiling water bath at 100 °C. After cooling to room temperature, added 2 ml of ethyl acetate and mixed. Then 1 ml of reagent A and 1 ml of sulphuric acid were added. The test tubes were kept at room temperature for 10 min and the intensity of red color developed was measured at 430 nm. A standard curve of saponin was run in the range of (10-40 µg). The content of saponins was expressed as g/100 g DW tissue.

3.2.4 Antioxidant parameters

3.2.4.1 Chlorophyll and carotenoids in fruits (Barnes *et al* 1992)

Principle: Chlorophyll and carotenoids are extracted in DMSO and absorbances are taken at 663, 645 and 480 nm. Using absorption coefficients, the amount of chlorophyll and carotenoids is calculated.

Reagents

i. Dimethyl sulphoxide (DMSO).

Procedure

Took 0.1 g of tissue and dipped in 5ml DMSO. The samples were kept in water bath at 60º C for 1 h for pigment extraction. The absorbance was read at 480, 645 and 663 nm. Total chlorophyll, chlorophyll a, chlorophyll b, and carotenoid content were calculated using given formulas and the contents were expressed as mg/g FW tissue

$$\text{Total chlorophyll} = \frac{20.2 \times A_{645} + 8.02 \times A_{665} \times V}{a \times 1000 \times W}$$

$$\text{Chl a} = \frac{12.47 \times A_{663} - 3.62 \times A_{645} \times V}{1000 \times W}$$

$$\text{Chl b} = \frac{25.06 \times A_{645} - 6.5 \times A_{663} \times V}{1000 \times W}$$

$$\text{Carotenoids} = \frac{1000 \times A_{480} - 1.29 \times \text{Chl a} - 53.78 \times \text{Chl b} \times V}{220 \times 1000 \times W}$$

Where W= Fresh weight of sample in grams, V= Volume of extract, a= Path length of light in the cell (1 cm), A_{480}, A_{645} and A_{663} are absorbance of samples at 480, 645 and 663 nm respectively**.**

3.2.4.2 Carotenoid content in seeds (Guizhen *et al* 2005)

Reagents

i. Petroleum ether (60°-80° C).

ii. Acetone.

Procedure

Took 0.2 g of seed flour in titration flask and added 9 ml of solvent mixture (petroleum ether: acetone :: 1:1). Kept on shaker at 100 rpm in dark for 6 h. Thereafter centrifuged at 2000 rpm for 10 min. Took the supernatant and read the absorbance at 445 nm against petroleum ether : acetone (1:1) as blank.

$$\text{Carotenoids (mg/100 g)} = \frac{A.Y\,(ml).10^3}{A\%_{1cm}\,g}$$

A= Highest Absorbance at 445nm,

Y= quantity of extraction solution,

g= sample weight

$A\%_{1cm}$= average absorption coefficient 2500 of carotenoid molecule

3.2.4.3 Total phenols (Swain and Hills 1959)

Principle: Phenols react with phosphomolybdic acid in Folin-Ciocalteau phenol reagent in alkaline medium and produce blue coloured complex (molybdenum blue) which is read spectrophotometrically at 760 nm.

Reagents

i. 80% aqueous methanol: Took 80 ml of methanol and made the volume to 100 ml with DDW.

ii. Saturated solution of sodium carbonate: Dissolved 25 g of anhydrous sodium carbonate in 50 ml of DDW by heating on water bath at 70-80°c. Kept the contents overnight and used the supernatant.

iii. Folin Ciocalteau phenol reagent diluted 1:1 (v/v) with distilled water.

iv. Standard gallic acid (100µg/ml): 10 mg of gallic acid was dissolved in 100 ml distilled water.

Procedure

Homogenised 0.3 g of tissue in 5 ml of 80% methanol and refluxed on water bath at 60° C for 1 h. The contents were filtered, evaporated and final volume was made to 10 ml with DDW. Took 0.5 ml of extract and added 2.5 ml of DDW. Subsequently added 0.5 ml of Folin phenol reagent (1:1 diluted) and after 5 min 1 ml of saturated sodium carbonate solution was added, vortexed and kept at room temperature for 30 min. The blue color developed was read at 760 nm against reagent blank. Gallic acid was used as standard in the range of 10-60µg. Content of total phenols was expressed as g/100 g DW tissue

3.2.4.4 O-dihydroxyphenols (Nair and Vaidyanathan 1964)

Reagents

i. 10% TCA: 10 g of TCA was dissolved in 100 ml of DDW.

ii. 0.5% Sodium nitrite: 0.5 g of sodium nitrite was dissolved in 100 ml of DDW. Prepared fresh before use.

iii. 0.5 N HCl: Took 1.82 ml of HCl and volume was made to 100 ml with DDW.

iv. 0.5 N NaOH: Dissolved 2 g of NaOH in 80 ml DDW and final volume was made to 100 ml with DDW.

v. Sodium Tungstate.

vi. Standard catechol (100 µg/ml): 10 mg of catechol was dissolved in 80 ml DDW and final volume was made to 100 ml with DDW.

Procedure

To 1 ml of extract (obtained in case of total phenols), added 0.5 ml of 10% TCA followed by 1 ml of sodium tungstate, 0.5 ml of 0.5 N hydrochloric acid and 1 ml of sodium nitrite solution. Mixed and after 5 min added 2 ml of 0.5 N NaOH solution. Took the absorbance at 540 nm against blank after 15 min. Standard of catechol was run in range of 10-100 µg. Content of ortho-dihyroxyphenol was expressed as g/100 g DW tissue.

3.2.4.5 Flavanols (Balabaa *et al* 1974)

Reagents

i. 0.1 M methanolic aluminium chloride: 1.33g of aluminium chloride was dissolved in 80 ml of methanol and the final volume was made to100 ml with methanol.

Procedure

To 1 ml of supernatant (obtained in case of total phenols), added 1 ml of water and 3 ml of methanolic aluminium chloride. Kept at room temperature for 1 h. The absorbance was recorded at 420 nm. Standard of rutin was run in range of 10-50 µg. Flavanol content was expressed as g/100 g DW tissue.

3.2.4.6 Tocopherols (Kayden *et al* 1973)

Principle: Vitamin E reduces ferric ions to ferrous ions which combine with bathophenanthroline to form an orange coloured complex which is read at 536nm.

Reagents

i. Absolute ethanol.
ii. Xylene.
iii. Bathophenanthroline reagent (0.4%): 0.4 g of Bathophenanthroline was dissolved in 100 ml of 95% absolute ethanol.
iv. Ferric chloride reagent: 60 mg of $FeCl_3.6H_2O$ was dissolved in 100 ml of 95% absolute ethanol.
v. Orthophosphoric acid: 0.5 ml of 85% orthophosphoric acid was dissolved in 100 ml of 95% absolute ethanol.
vi. Standard α-Tocopherol (0.02 mg/ml): Dissolved 2 mg of α-Tocopherol in 100 ml of 95% absolute ethanol).

Procedure

Homogenised 0.3 g of tissue in 2 ml of ethanol and centrifuged the contents at 4000 rpm for 10 min. Pipetted out 0.5 ml of supernatant, standard Vitamin E and absolute ethanol, taken individually in stoppered glass centrifuge tubes and marked as test, standard and blank respectively. To each tube, added 0.5 ml of distilled water. Vortexed and then added 0.5 ml of purified xylene and vortexed again for 2 min and centrifuged at 5000 rpm for 5 min. 0.2 ml of xylene layer was carefully pipetted out from each tube into another set of freshly labelled stoppered glass test tubes and added 0.6 ml of xylene to make volume to 0.8 ml. Added 0.4 ml bathophenanthroline reagent and mixed thoroughly. Added 0.4 ml ferric chloride reagent and 0.4 ml of orthophosphoric acid. Cyclomixed the contents after each addition. Absorbance of test, standard and blank was read at 536 nm against xylene as reference within 30 sec. Sample should not come in contact with water and sunlight. Tocopherol content was expressed as mg/100 g FW tissue

3.2.4.7 Ascorbic acid (Jagota and Dani 1982)

Principle: Ascorbic acid react with Folin reagent forming a blue colored complex whose absorbance is read at 760 nm. The developed color is stable upto 18 h.

Reagents

i. 10% TCA: 10 g TCA was dissolved in 100 ml of DDW.

ii. Folin Ciocalteu reagent: Folin Ciocalteu reagent and DDW were mixed in the ratio of 1:9. It was prepared fresh.

Procedure

Homogenised 0.1 g of tissue in 2 ml of 10% TCA solution. The homogenate was centrifuged at 10,000 rpm for 10 min. To 1 ml of supernatant added 1 ml of TCA and 0.2 ml of Folin Ciocalteu reagent. The reaction mixture was incubated at 37º C for 20 min. Absorbance of blue color developed was recorded at 760 nm against blank. Standard was run in the range of 10-60µg. The content of ascorbic acid was expressed as mg/100 g FW tissue.

3.2.4.8 Total antioxidant activity

3.2.4.8.1 DPPH Activity (Blois 1958)

Principle: The hydrogen atom of an antioxidant present in sample reduce the odd electron of nitrogen in DPPH to corresponding hydrazine that absorb at 517 nm.

Reagents

i. Methanol.

ii. DPPH (0.1mM in methanol): Dissolved 0.004 g of DPPH in 80 ml and then made the volume to100 ml with methanol.

Procedure

Homogenised 0.1 g of tissue in 2 ml of methanol. The homogenate was centrifuged at 10,000 rpm for 10 min. To 10 µl of supernatant added 990 µl of methanol and 1ml of DPPH solution. Mixed and incubated in dark for 30 min at room temperature. Absorbance of blank (methanol), sample and control (methanol + DPPH) was recorded at 517 nm. Total antioxidant activity was expressed as % of DPPH radical scavenged. The annihilation activity of free radicals was calculated as % inhibition according to the following formula

% Inhibition = (Ac- As)/Ac X 100

Where: Ac- Absorbance of control solution.

As- Absorbance of test solution.

3.2.4.8.2 FRAP Activity (Benzie and Strain 1996)

Principle: FRAP assay is based on rapid reduction in ferric–tripyridyltriazine by antioxidant present in sample forming ferrous- tripyridyltriazine, a blue colored product.

Reagents

i. 200 mM Sodium phosphate buffer (pH 6.6): Dissolved 3.12 g of monobasic sodium phosphate in minimum quantity of DDW and made the volume to 100 ml with DDW (X solution). Dissolved 1.77g of dibasic sodium phosphate in minimum quantity of DDW and final volume was made to 50 ml with DDW (Y solution). Mixed 62.5 ml of X solution and 37.5 ml of Y solution to have 200 mM sodium phosphate buffer (pH 6.6).

ii. 1% Potassium ferricyanide: 1 g of potassium ferricyanide was dissolved in 100 ml of DDW.

iii. 10% TCA: 10 g of TCA was dissolved in 100 ml of DDW.

iv. 0.1% Ferric chloride: Add 0.1 g of ferric chloride in 100 ml of DDW.

Procedure

Homogenized 0.2 g tissue in 2 ml of methanol and centrifuged at 10,000 rpm for 10 min. To 0.5 ml of supernatant added 0.5 ml of phosphate buffer and 0.5 ml of potassium ferricyanide solution. Test tubes were kept in water bath at 50^{o} C for 20 min. Added 0.5ml of 10% TCA solution and again centrifuged the contents (if precipitates were formed). Took 1 ml of supernatant and added 1ml distilled water and 0.1ml of ferric chloride solution. After 5 min, absorbance was recorded at 700 nm. Standard of vitamin C was run in the range of 10-100 µg. Total antioxidant activity was expressed as mg/g AAE.

Statistical Analysis

The data was calculated as the mean of triplicates. Significance among *Abelmoschus* genotypes and accessions for various parameters was calculated with CRD ANOVA and Tukey's *post havoc* test using CPCS-1 and SPSS softwares.

CHAPTER IV

RESULTS AND DISCUSSION

The wild relatives are the treasure trove of traits for crop improvement and dietary diversification (Singh and Abhilashn 2017). The biochemical characterisation of wild germplasm is a pre-requisite for the crop improvement programmes. In the present study ten wild *Abelmoschus* species were evaluated for variation in biochemical traits in immature pods and mature seeds. The results of the study are discussed under following headings.

4.1 Biochemical characterisation of immature pods

4.1.1 Nutrients

4.1.1.1 Carbohydrates

4.1.1.2 Moisture and Ash

4.1.1.3 Proteins and amino acids

4.1.1.4 Antioxidants

4.1.2 Antinutrients

4.2 Biochemical characterisation of mature seeds

4.2.1 Proteins

4.2.2 Oil content and Fatty acid profile

4.2.3 Carotenoids and Tocopherols

4.2.4 Minerals

4.1 Biochemical characterisation of immature pods

The immature pods of cultivated okra have been reported to be endowed with carbohydrates, minerals, vitamins and antioxidant properties (Gemede *et al* 2015). The cultivated okra is vulnerable to biotic and abiotic stresses; the impediments in realising full potential of the crop (Lokesh 2017). Wild relatives are repositories of traits that make them resilient to environmental vagaries and also offer nutritional superiority. The biochemical evaluation of wild relatives of a crop is important for paving way for crop improvement programmes. Wild relatives were first time used for improvement in sugar cane in the 20th century (Plucknett *et al* 1987) and many more crops followed the trend. In the present study wild *Abelmoschus* species were evaluated for nutrient, anti-nutrient and antioxidant composition.

4.1.1 Nutrients

4.1.1.1 Carbohydrates

Carbohydrates are most abundant and diverse class of organic compounds that include total soluble sugars, reducing sugars, non-reducing sugars, mucilage and fibers.

Besides being source of energy, carbohydrates form 90% of sap solute molecules which translocate from source to sink during the fruit formation process. Okra pods are good source of carbohydrates. The major carbohydrates reported in cultivated okra pods include cellulose, starch, sugars, mucilage and fiber (Kumar *et al* 2009, Kumar *et al* 2010). Total carbohydrate content in immature pods of wild okra genotypes under study varied from 22.34- 93.10% on DW basis with mean value of 56.52% (Table 4.1). Maximum content of carbohydrates was found in *Abelmoschus moschatus* IC 470737 (93.10%) and *Abelmoschus tetraphyllus* (92.98%) (Table 4.1). The cultivated genotype Punjab Padmini depicted 88.25% which was less than that in *Abelmoschus moschatus* IC 470737 and *Abelmoschus tetraphyllus*. Mohite and Gurav (2019) reported the carbohydrate content in wild okra to range from 39.20% in *A.manihot* to 28.38% in *A. ficulneus*. Gemede *et al* (2016) reported the range from 36.66- 50.97% in eight okra cultivated genotypes. Roy *et al* (2014) observed a content of 7.03% in okra pods on fresh weight basis. Kouassi *et al* (2013) reported the range from 65.24% for the Dioula variety of okra and 63.06 to 65.53% with an average of 63.92% for the Baoule variety. Ndlovu and Afolayan (2007) analysed wild okra *Chorchorus olitorius* from South Africa for carbohydrate content and reported mean value of 695g/kg^{-1} immature pods. Other studies reported carbohydrate content of 6.4- 15.8g/100 g on FW basis (Kumar *et al* 2013, Sachan *et al* 2017, Petropoulos *et al* 2018).

Total soluble sugars, Reducing sugars and Non-Reducing sugars

Total soluble sugars in wild okra immature pods ranged from 13.74- 84.36% on DW basis. *A. moschatus* (IC 470737) registered 84.36% while the lowest content was observed in *A. tuberculatus* 13.74%. Two accessions *viz*. IC 141056, IC 470737, and *A. tetraphyllus* had total soluble sugar content > 50%, five genotypes registered a range of 20- 41.83% while Punjab Padmini had 68.96% DW. Reducing sugars registered variation of 0.17- 19.41% on DW basis with the mean value of 6.79% while the non-reducing sugar content ranged from 13.47- 64.95% on DW basis with average of 33.10%. Cultivated variety had higher content of reducing sugar (10.69%) and non-reducing sugars (58.27%) in comparison to average of wild genotypes. *A. moschatus* accession IC 470737 exhibited maximum content of reducing as well as non-reducing sugars with value of 19.41% and 64.95% respectively. *A. moschatus* accession IC 470737 represented maximum amount of total carbohydrates, total soluble sugars as well as reducing sugar and non-reducing sugars. Petrolpoulos *et al* (2018) reported total soluble sugar content in the range of 2.07- 4.3 g/100 g FW in mediterranean okra genotypes. Chanchal *et al* (2018) reported a range of 1.2- 5.8% on FW basis in cultivated okra pods.

Table 4.1 Variation in total carbohydrates and related components in immature pods of wild okra

Genotypes		Biochemical Parameters					
		Total carbohydrate (g/100 g DW)	Total soluble sugars (g/100 g DW)	Reducing sugars (g/100 g DW)	Non reducing sugars (g/100 g DW)	Mucilage (g/100 g DW)	Crude fiber (g/100 g DW)
Abelmoschus moschatus	IC 141056	62.85 ± 0.19^{d}	53.95 ± 0.39^{d}	10.89 ± 0.02^{c}	43.06 ± 0.37^{c}	10.27 ± 0.20^{g}	26.36 ± 0.41^{c}
	IC 140986	69.38 ± 0.29^{c}	41.83 ± 0.03^{e}	3.72 ± 0.02^{h}	38.11 ± 0.05^{e}	7.41 ± 0.07^{i}	16.42 ± 0.33^{e}
	IC 140985	62.88 ± 0.19^{d}	29.68 ± 0.07^{h}	4.32 ± 0.10^{g}	25.36 ± 0.18^{h}	6.64 ± 0.15^{j}	18.17 ± 0.24^{d}
	IC 470737	93.10 ± 0.20^{a}	84.36 ± 0.17^{a}	19.41 ± 0.11^{a}	64.95 ± 0.23^{a}	16.07 ± 0.02^{d}	26.32 ± 0.32^{c}
Abelmoschus mizonagenesis		45.73 ± 0.15^{f}	33.10 ± 0.01^{g}	4.71 ± 0.06^{f}	28.39 ± 0.05^{g}	13.84 ± 0.56^{e}	27.28 ± 0.20^{b}
Abelmoschus tetraphyllus		92.98 ± 0.07^{a}	60.65 ± 0.09^{c}	18.70 ± 0.07^{b}	41.95 ± 0.05^{d}	8.75 ± 0.03^{h}	26.29 ± 0.23^{c}
***Abelmoschus angulosus var.grandiflorus* IC 203833**		51.88 ± 0.07^{e}	36.52 ± 0.06^{f}	5.27 ± 0.04^{e}	31.25 ± 0.10^{f}	13.24 ± 0.01^{f}	29.53 ± 0.12^{a}
Abelmoschus tuberculatus		22.34 ± 0.09^{i}	13.74 ± 0.22^{k}	0.27 ± 0.02^{j}	13.47 ± 0.21^{k}	18.81 ± 0.05^{b}	16.18 ± 0.09^{e}
Abelmoschus manihot	–	29.90 ± 0.28^{h}	21.65 ± 0.21^{j}	0.17 ± 0.04^{j}	21.48 ± 0.21^{j}	17.11 ± 0.06^{c}	11.41 ± 0.05^{g}
	IC 90339	34.22 ± 0.11^{g}	23.48 ± 0.21^{i}	0.45 ± 0.01^{i}	23.03 ± 0.37^{i}	19.64 ± 0.04^{a}	14.55 ± 0.04^{f}
Average		56.52	39.90	6.79	33.10	13.18	20.25
Abelmoschus esculentus	Punjab Padmini	88.25 ± 0.22^{b}	68.96 ± 0.09^{b}	10.69 ± 0.04^{d}	58.27 ± 0.13^{b}	6.56 ± 0.03^{j}	16.86 ± 0.07^{e}
CD (5%)		0.23	0.19	0.12	0.23	0.14	0.28

Each value is a mean ± SD of three replications

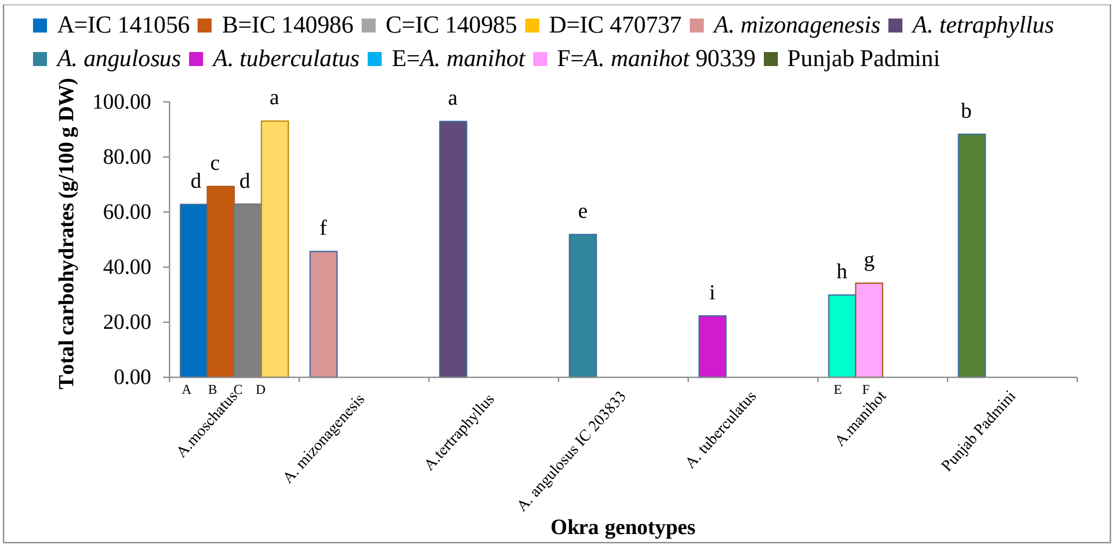

Fig. 4.1(a) Variation in total carbohydrates in immature pods of wild okra

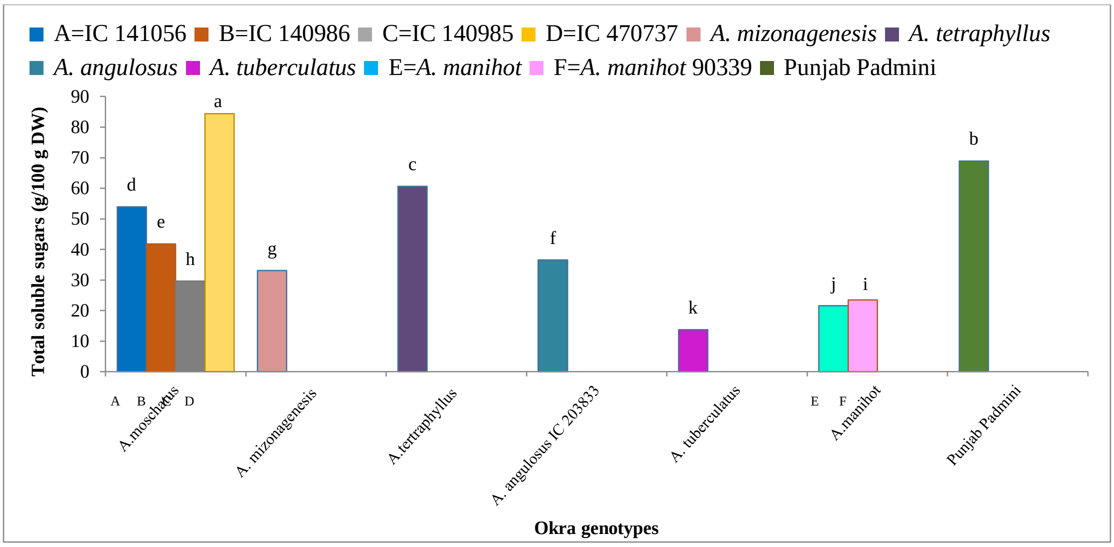

Fig. 4.1(b) Variation in total soluble sugar content in immature pods of wild okra

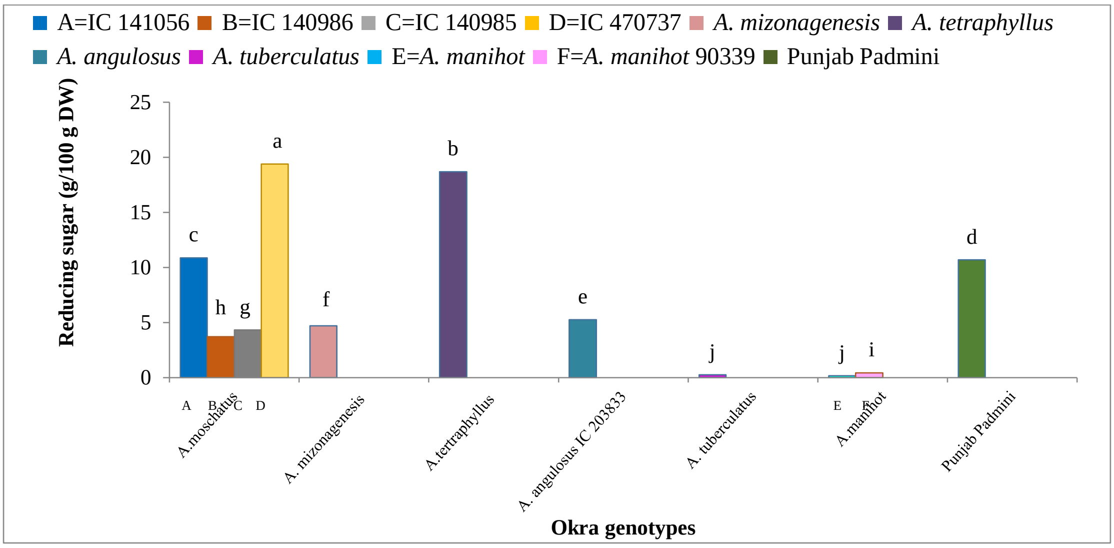

Fig. 4.1(c) Variation in reducing sugar content in immature pods of wild okra

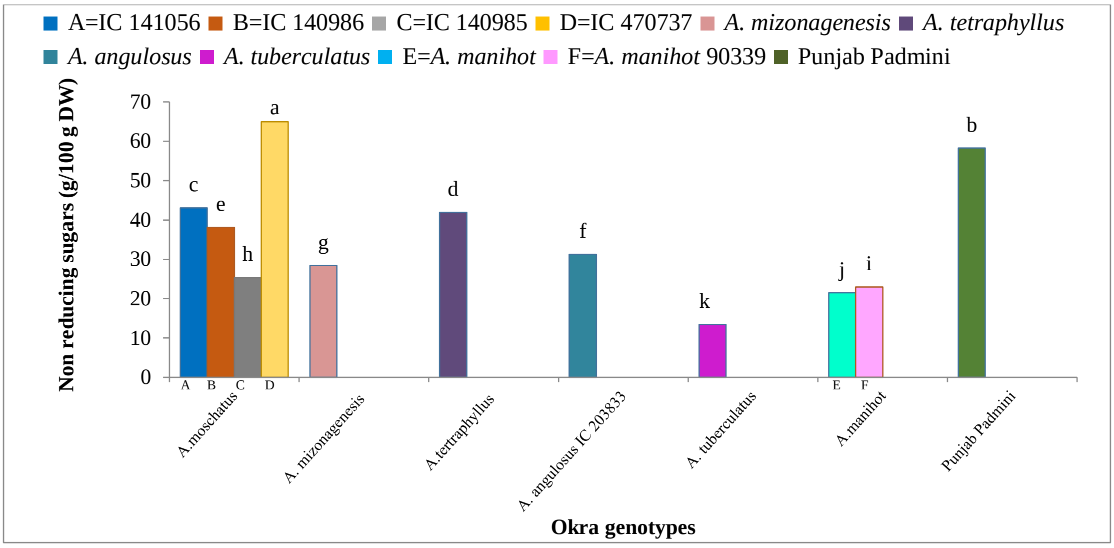

Fig. 4.1(d) Variation in non-reducing sugar content in immature pods of wild okra

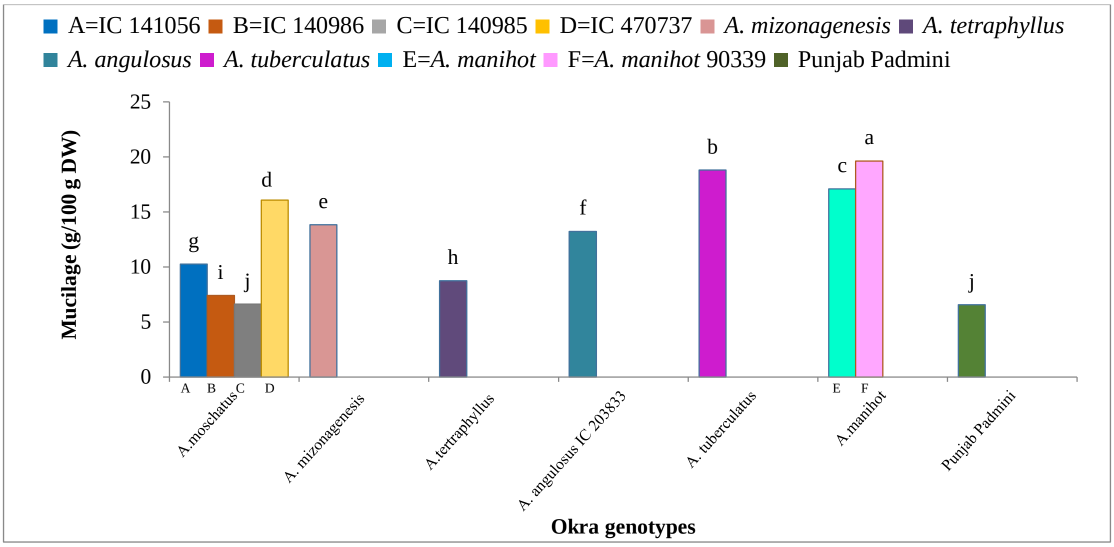

Fig. 4.1(e) Variation in mucilage content in immature pods of wild okra

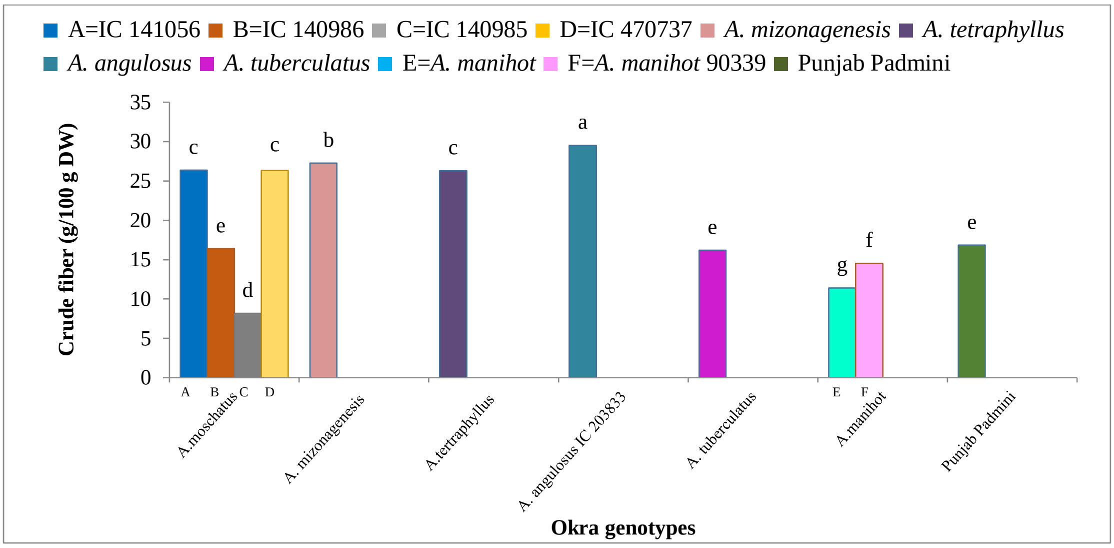

Fig. 4.1(f) Variation in crude fiber content in immature pods of wild okram

Dubey *et al* (2013) reported total soluble sugar content of 0.363mg/g FW and 0.648mg/g FW in two ambrette germplasm of *A. moschatus*. Sandhi *et al* (2017) reported that wild genotypes of okra *(Abelmoschus tetraphyllus, Abelmoschus angulosus and Abelmoschus moschatus*) registered total soluble sugar content of 15.21– 18.36 mg/g FW and reducing sugar content in the range of 2.76- 5.75 mg/g FW.

Roy *et al* (2014) observed total soluble sugar content of 1.2%, sucrose content of 0.40% and starch 0.34% in fresh pods of cultivated okra. Fermin *et al* (2018) reported that reducing sugar content increased significantly to 0.69 mg / 100g DW at 15th day of maturity and then decreased to 0.64 mg / 100g DW with increase in maturity upto 20th day for Koto variety of okra but in Tomi variety, the levels increased from 0.13 mg / 100g to 0.7mg / 100g DW at 15th day of maturity and then decreased to 0.56 mg / 100g DW at 20 days of maturity. Similarly the total soluble sugar content increased from 2.49 to 4.21 mg / 100g DW (from 5th to 15th day of maturity) in koto variety and from 2.06 to 5.86 mg / 100g DW (from 5th to 15th day of maturity) in Tomi variety and decreased at 20th day in both the varieties.

Mucilage

Okra contain carbohydrates mainly in form of mucilage. Mucilage is a complex polysaccaharide formed of sugars and uronic acids in association with proteins, carbohydrates, neutral sugars and minerals (Ahiakpa *et al* 2014). The glycans present in mucilage increase the viscosity of aqueous suspensions (Kumar *et al* 2010). Okra mucilage is used as additives in the formulation of flour-based adhesives and in clarifying sugarcane juice. It also act as an emulsifier, thickener, gelling agent, or texture modifier (Noorlaila, Siti Aziah, Asmeda, & Norizzah, 2015). Mucilage also contains anticancer, antimicrobial, hypoglycemic, anti-ulcer activities (Mohite and Gurav 2020). It is used for plasma replacement or as blood expander (Madison D 2008 and Maramag RP 2013). Okra typically differs from most other common vegetables in having high mucilage content.

The green pods of wild okra depicted mucilage in the range of 6.64 (*A. moschatus* accessions IC 140985)- 19.64% (*A.manihot* (90339)) on DW basis while the cultivated okra (Punjab Padmini) had content of 6.56% (Table 4.1). The wild genotypes; *A.moschatus* (IC 141056 and IC 470737), *A. mizonagenesis*, *A. angulosus* (IC 203833), *A. tuberculatus* and *A. manihot* possessed mucilage content of 10.27%, 16.07%, 13.84%, 13.24%, 18.81% and 17.11% respectively. Nair (2013) reported that the mucilage content in three wild okra genotypes *A. moschatus, A. angulosus* and *A. manihot* ranged from 0.109- 0.357 g/g, 0.102- 0.194 g/g, 0.125- 0.382 g/g respectively at different stages of fruit development. Gemede *et al* (2018) reported the mucilage content of 1.25 to 3.45 g/100 g on fresh weight basis in the pods of eight cultivated okra accessions grown in Benishangul-Gumuz region, Western Ethiopia.

Kaur *et al* (2015) reported the content in cultivated okra fruit to lie in the range of 2.68 - 5.23%. Sharma *et al* (2013) and Ahiakpa *et al* (2014) reported mucilage content in twenty one genotypes of cultivated okra to be in the range of 5.3-37.67% .

Crude fiber

Okra is a vegetable crop which is low in calories with practically no fat but high in fiber content. Its immature pods are considered to be a rich source of crude fiber. Soluble okra fiber is mainly present as gums and pectins. Pectin is important dietary component that helps to lower cholesterol level and provide protection against cardiovascular disease (Ngoc *et al* 2008, Sengkhamparn *et al* 2009) and also keeps the blood sugar level under control (Kumar *et al* 2013). Okra fiber maintains colon health and ensure proper intestinal functionality (Georgiadisa *et al* 2011)

Table (4.1) also represent crude fiber content in okra genotypes. It varied from 11.41-29.53% on DW basis with mean value of 20.25% in wild okra green pods. *A. moschatus* (IC 141056, IC 470737), *A. mizonagenesis*, *A. tetraphyllus* were at the par and in *A. moschatus* (IC 140986), *A. tuberculatus, A.manihot* (90339) genotypes the value ranged from 16-20%. Maximum content (29.53%) was found in *A. angulosus var. grandiflorus* IC 203833. Most of the wild genotypes had higher content than the cultivated genotype (Punjab Padmini). Mohite and Gurav (2019) reported the average fiber content in wild okra species *A. ficulneus* and *A. manihot* to be 23.49% and 22.90% respectively. Ehile *et al* (2018) observed the fiber content of 18.6% DW in *A. tuberculatus*. Chanchal *et al* (2018) reported content of 3.2g/100g in fresh fruits of cultivated okra. Gemede *et al* (2016) reported 11.97-29.93% fiber in cultivated okra fresh fruits. Adamma *et al* (2014) reported fiber content of 14.46% in vegetative part and 29.76% in fruits. Ndamitso *et al* (2012) observed 9.46% crude fiber in pods of *Abelmoschus esculentus*. Ndlovu and Afolayan (2008) reported 35.50 g/kg^{-1} of crude fiber content in immature fruit of *Chorchorus olitorius.* Epidemiological evidences also suggest that high fiber in diet may contribute to decrease in incidence of diseases like diabetes, cardiac disease, colon cancer, obesity and various GI disorders (Tucker and Thomas 2009, Park *et al* 2009).

4.1.1.2 Dry matter and Ash

The dry matter of food include carbohydrates, proteins, vitamins, minerals and antioxidants. Carbohydrates, fats and proteins provide energy in food which make up 90% of the dry weight of diet. Dry matter content in wild okra immature fruits ranged from 11.18-27.59% on FW basis with average value of 18.29%. Punjab Padmini contained dry matter content of 10.41%. *A. moschatus* IC 470737 had minimum dry matter content (11.18%) but highest was found in *A. tuberculatus* (27.59%). Four wild genotypes namely *Abelmoschus angulosus var.grandiflorus* IC 203833, *A. tuberculatus*, *A. manihot*, *A. manihot sp.*IC 90339 had dry matter content >20%. Gemede *et al* (2016) reported dry matter content of 9.69-

Table 4.2 Variation in dry matter and ash content in immature pods of wild okra

Genotypes		**Biochemical Parameters**	
		Dry matter (%)	**Ash (% FW)**
Abelmoschus moschatus	IC 141056	15.54 ± 0.41[e]	5.50 ± 0.09[b]
	IC 140986	15.28 ± 0.32[e]	4.65 ± 0.43[c]
	IC 140985	17.30 ± 0.32[d]	4.04 ± 0.15[cd]
	IC 470737	11.18 ± 0.13[f]	3.78 ± 0.20[d]
Abelmoschus mizonagenesis		15.05 ± 0.05[e]	6.83 ± 0.23[a]
Abelmoschus tetraphyllus		11.70 ± 0.18[f]	1.10 ± 0.17[e]
***Abelmoschus angulosus var.grandiflorus* IC 203833**		23.57 ± 0.14[b]	5.73 ± 0.29[b]
Abelmoschus tuberculatus		27.59 ± 0.08[a]	5.60 ± 0.21[b]
Abelmoschus manihot	–	21.97 ± 0.02[c]	3.88 ± 0.24[d]
	Abelmoschus manihot sp. IC 90339	23.80 ± 0.11[b]	6.94 ± 0.13[a]
Average		18.29	4.80
Abelmoschus esculentus	Punjab Padmini	10.41 ± 0.10[g]	5.79 ± 0.25[b]
CD (5%)		0.27	0.56

Each value is a mean ± SD of three replications

13.33%. Kouassi *et al* (2013) reported the average dry matter content of 7.33 % in Dioula variety of okra and 7.28% in Baoule variety.

Ash content

Ash is an inorganic residue that remains after the complete combustion of any organic material at very high temperature (600°C). It is an important component in proximate analysis of biological material and mainly consists of metal oxides and minerals like sodium, potassium, calcium and magnesium as major elements and iron, copper, aluminium, manganese or zinc, iodine, fluorine, arsenic as minor elements. Okra have high content of ash indicating it as a good source of minerals. Ash content in wild pods ranged from 1.10- 6.94% on FW basis with mean value of 4.80% (Table 4.2). The average ash content of wild genotypes (4.80%) was less than that in cultivated okra; Punjab Padmini (5.79%). *A.mizonagenesis* and *A.manihot* accession IC 90339 had content greater than Punjab Padmini i.e 6.83% and 6.94% respectively. Ndlovu and Afolayan (2008) reported 57.40 g/kg^{-1} of ash

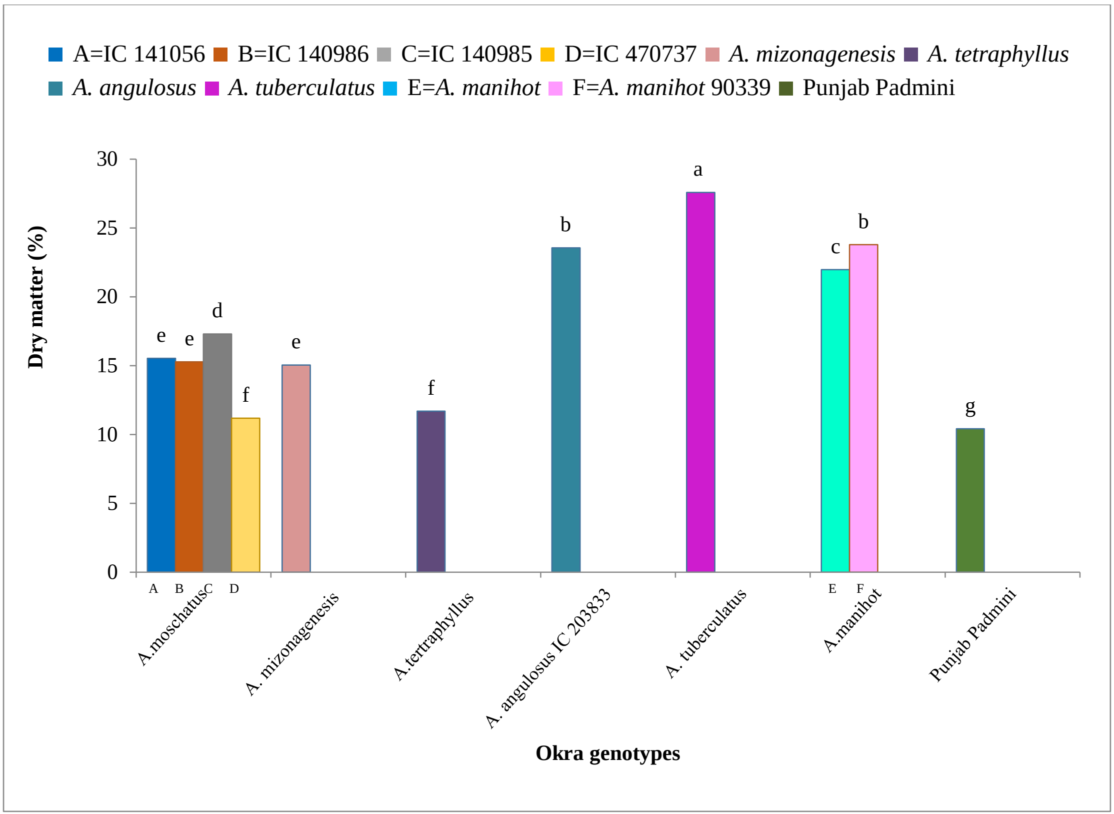

Fig. 4.2(a) Variation in dry matter content in immature pods of wild okra

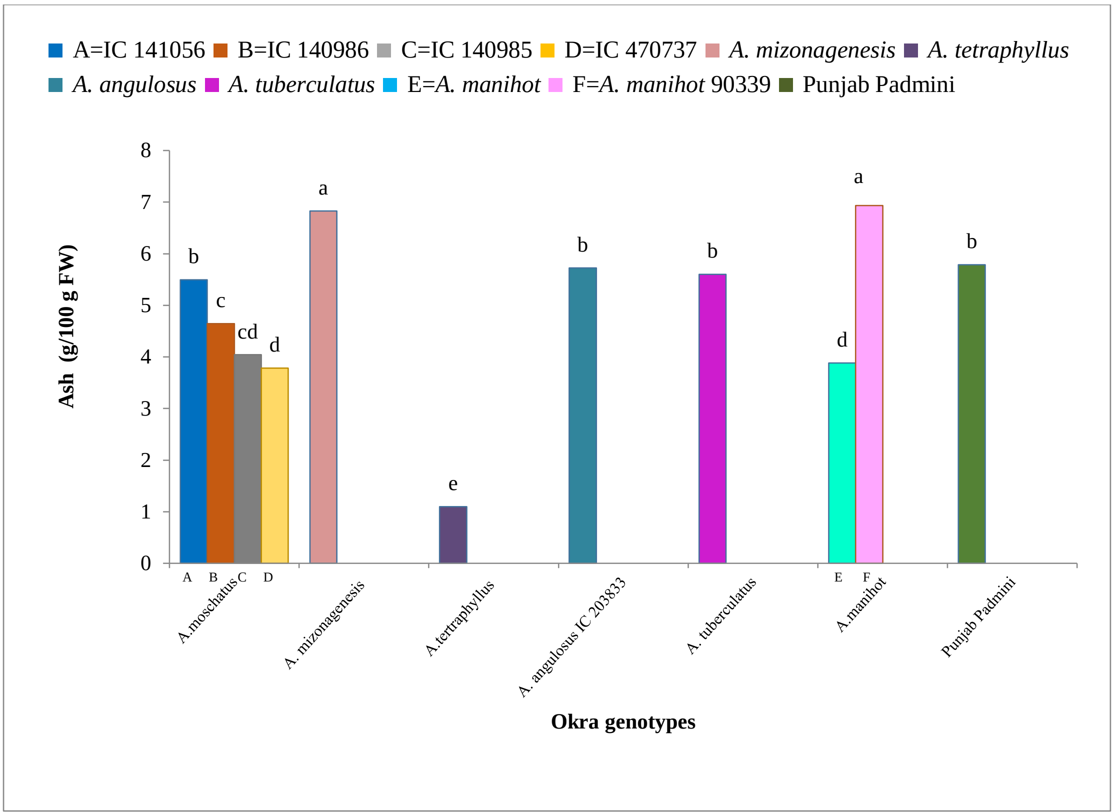

Fig. 4.2(b) Variation in ash content in immature pods of wild okra

content in immature fruit of *Chorchorus olitorius.* Ndamitso *et al* (2012) reported 7.75% on FW basis in *Abelmoschus esculentus* immature pods. Nair and Fahsa (2013) reported ash content in *A.angulosus, A.manihot* and *A.moschatus* to be 6.3%, 5.6% and 4.8% DW of FW inpods respectively. Farooq *et al* (2013) calculated ash content to characterize mucilage and found total ash value of 7.53%, acid insoluble ash value of 0.93% and water soluble ash value of 4.0%. Gemede *et al* (2015) reported 5.37- 11.30% ash in okra pods. Petropoulos *et al* (2018) registered the ash content in mediterranean okra genotypes to be from 0.66- 0.97 g/100 g FW and 0.10- 0.94 g/100 g FW in small and large fruit respectively. Mohite and Gurav (2019) reported ash content of 22.29% DW and 19.62% on DW basis in pods of *A.manihot* and *A.ficulneus* respectively.

4.1.1.3 Proteins and amino acids

Proteins are important for human growth and play essential role in all biological processes. They act as catalyst, provide mechanical support, protection etc. The amino acid content, their proportion, and digestibility in humans characterize a protein's biological value (Ewa *et al* 2011).

The data in Table (4.3) indicated that crude protein, total soluble protein and free amino acid content varied significantly among different wild okra species. The crude protein in wild pods varied from 2.13- 6.32% on FW basis with average value of 3.47% while the content in Punjab Padmini was 4.86%. All the accessions of *A.moschatus* were at par except IC 140986 which had 4.18% of crude protein. Total soluble protein range detected was 0.93- 3.56% on FW basis and the average was 1.73% (Table 4.3). *A.moschatus* (IC 140986, IC140985), *A. tetraphyllus and A. angulosus var grandiflorus* IC 203833 were at par for total soluble protein content and in *A. tuberculatus, A. manihot, A. manihot* IC 90339 the content recorded was 2.10%, 3.56% and 2.16% respectively. The free amino acid content in wild okra pods depicted a range from 0.16- 0.27% on fresh weight basis. Punjab Padmini depicted value higher than wild okra. Maximun content was observed in *A. manihot.*

Sami *et al* (2013) reported eleven essential amino acids in immature pods of okra. The major amino acids registered range of 2.91– 4.92% for aspartic acid, 1.38- 2.53% for proline, 1.74- 2.44% for glutamic acid, 0.67- 1.44% for arginine, 0.56- 0.78% for leucine, 0.53- 0.71% for alanine, 0.50- 0.69% for lysine, 0.56-0.78% for serine and 0.36- 0.52% for phenylalanine. Ndlovu and Afolayan (2008) reported 76.50g/kg^{-1} crude protein content in fruits of *Chorchorus olitorius.* Ndamitso *et al* (2014) reported that in cultivated okra pods crude protein constituted 14.10% on fresh weight basis. Singh *et al* (2014) exhibited 2.10g/100 g of crude protein in cultivated okra pods. Gemede *et al* (2015) reported a range of 10.25-26.16% on dry weight basis for okra pods. Petropoulos *et al* (2018) observed that the

Table 4.3 Variation in crude protein, total soluble protein and free amino acids content in immature pods of wild okra

Genotypes		Biochemical Parameters		
		Crude protein (%FW)	Total soluble proteins (% FW)	Free amino acids (% FW)
Abelmoschus moschatus	IC 141056	2.66 ± 0.12^{g}	0.93 ± 0.05^{h}	0.18 ± 0.08^{e}
	IC 140986	4.18 ± 0.05^{c}	1.26 ± 0.08^{f}	0.16 ± 0.07^{f}
	IC 140985	2.13 ± 0.05^{h}	1.21 ± 0.04^{f}	0.18 ± 0.05^{de}
	IC 470737	2.90 ± 0.03^{f}	1.06 ± 0.15^{g}	0.19 ± 0.03^{d}
Abelmoschus mizonagenesis		3.30 ± 0.05^{e}	1.85 ± 0.28^{d}	0.16 ± 0.08^{f}
Abelmoschus tetraphyllus		2.76 ± 0.11^{fg}	1.54 ± 0.60^{e}	0.20 ± 0.04^{d}
***Abelmoschus angulosus var.grandiflorus* IC 203833**		2.85 ± 0.01^{fg}	1.59 ± 0.06^{e}	0.26 ± 0.01^{b}
Abelmoschus tuberculatus		3.75 ± 0.09^{d}	2.10 ± 0.48^{c}	0.22 ± 0.01^{c}
Abelmoschus manihot	–	6.32 ± 0.07^{a}	3.56 ± 0.14^{a}	0.27 ± 0.04^{a}
	Abelmoschus manihot sp. IC 90339	3.82 ± 0.06^{d}	2.16 ± 0.01^{c}	0.19 ± 0.02^{d}
Average		3.47	1.73	0.21
Abelmoschus esculentus	Punjab Padmini	4.86 ± 0.08^{b}	2.72 ± 0.08^{b}	0.27 ± 0.08^{ab}
CD (5%)		0.19	0.96	0.61

Each value is a mean ± SD of three replications

small green pods of mediterranean okra depicted crude protein content range of 1.88- 3.44 g/100 g on fresh weight basis but the large sized fruits exhibited a range of 1.37- 2.91 g/100 g FW in relation to harvest stage. Mohite and Gurav (2019) reported the value in *A. manihot* and *A.ficulneus* to be 5.47g/100g and 5.33g/100g respectively.

4.1.1.4 Antioxidants

Okra is an important vegetable crop with a diverse array of nutritional quality and potential health benefits. The major antioxidants present in okra are carotenoids, Vitamins C, Vitamin E, and phenolic compounds (Gemede *et al* 2014). Antioxidants scavange reactive oxygen species (ROS) and protect against their harmful effect.

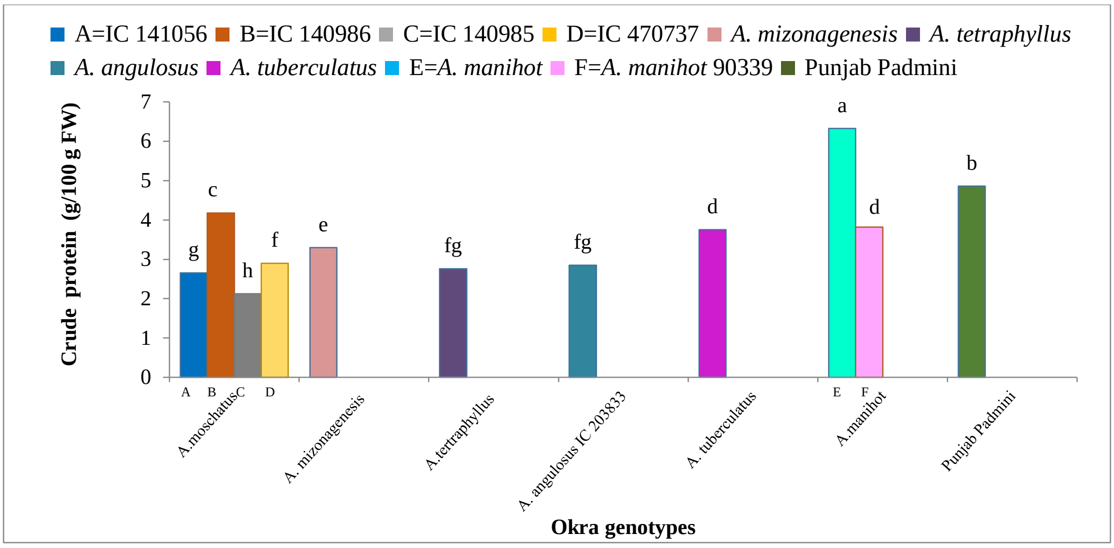

Fig. 4.3(a) Variation in crude protein content in immature pods of wild okra

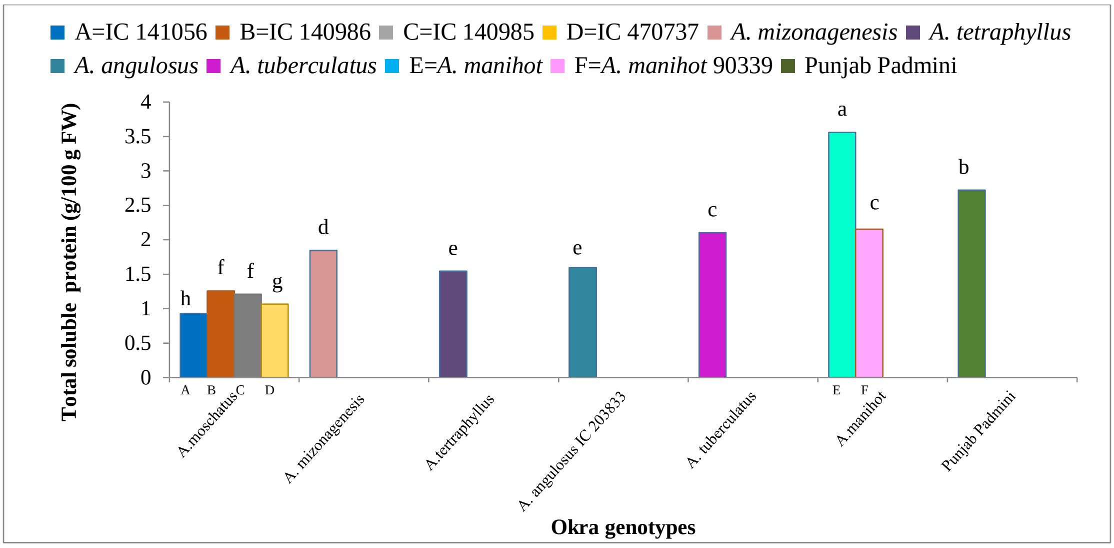

Fig. 4.3(b) Variation in total soluble protein in immature pods of wild okra

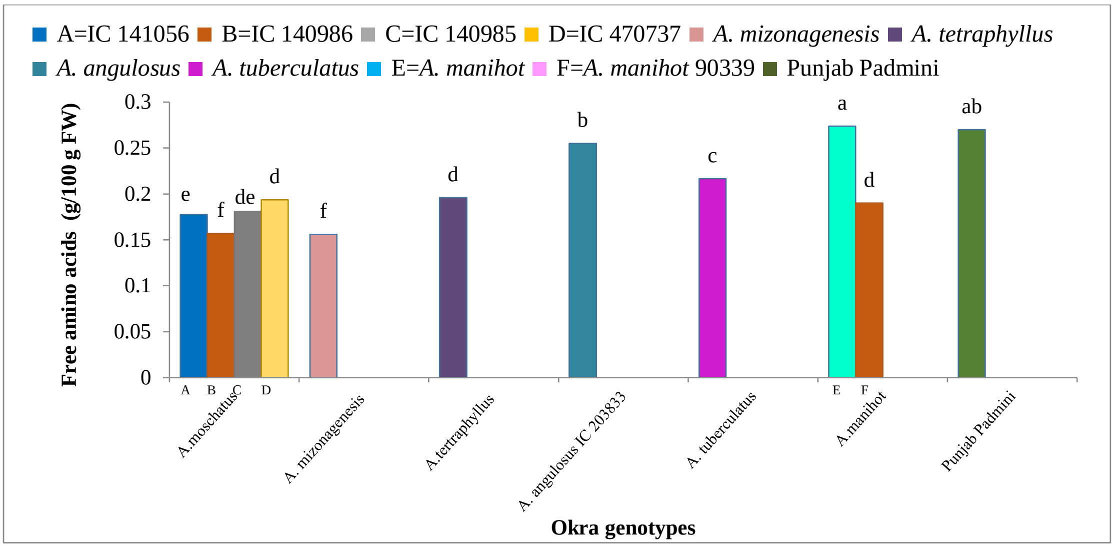

Fig. 4.3(c) Variation in free amino acid content in immature pods of wild okra

Total Chlorophyll and Carotenoids

Chlorophyll and carotenoids are important constituents of photosynthetic organelles of all higher plants. These photosynthetic pigments provide photosynthetic potential to plant (Xue & Yang 2009) and are also beneficial for human health. Chlorophyll has therapeutic properties and protect against cancer, cardiovascular disease, stimulate immune system, normalize blood pressure and prevent anemia (Znidarcic *et al* 2011) and carotenoids are precursor of vitamin A therein play role in vision, maintain intestinal mucous membrane and support digestive system to function properly. It also provide protection to lipid rich tissue.

The chlorophyll a content in the green pods of wild okra varied from 13.07- 38.63 mg/100g on fresh weight basis (Table 4.4). Chlorophyll b content ranged from 1.68- 9.04 mg/100 g FW and total chlorophyll content ranged from 15.84- 52.83 mg/100 g FW. *Abelmoschus manihot* and *Abelmoschus manihot sp.* accession IC 90339 had maximum content of chlorophyll a, chlorophyll b and total chlorophyll. Three accessions of *A.moschatus* namely IC 141056, IC 140986, IC 140985 were at par with each other except IC 470737 that registered higher value of all three chlorophylls. Punjab Padmini registered lower content of chlorophyll a (6.68 mg/100 g FW), chlorophyll b (0.69 mg/100gFW) and total chlorophyll (8.35 mg/100 g FW) than the average of wild genotypes. Total carotenoid content in wild pods ranged from 4.48- 13.25 mg/100 g FW (Table 4.4) while Punjab Padmini recorded 1.86 mg/100 g FW. In *A. moschatus* (accessions IC 141056, IC 140986, IC 140985, IC 470737), *A. mizonagenesis*, *A. tetraphyllus* and *A. angulosus var grandiflorus* IC 203833 the content ranged from 4.48- 5.78 mg/100 g FW. However highest content was observed in *A. manihot* (13.25 mg/100 g FW). Yora and Sabir (2018) reported that chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content in fruit of thirteen cultivated okra genotypes ranged from 0.036- 0.102 mg/100 g FW, 0.014- 0.041 mg/100 g FW, 0.051- 0.143 mg/100 g FW and 0.012- 0.034 mg/100 g FW respectively. Petropoulos *et al* (2018) reported 0.38- 1.43g/100 g FW of chlorophyll a, 0.21- 0.82g/100 g FW of chlorophyll b, 0.68- 2.25 g/100 g FW of total chlorophyll, 0.017- 0.30 g/100gmFW of β carotene in small and 0.31- 1.51g/100gm FW of chlorophyll a, 0.17-0.87 g/100gm FW of chlorophyll b, 0.55- 2.38 g/100gmFW of total chlorophyll and 0.015-0.31 g/100gmFW of β carotene in large sized fruit of mediterranean okra. Gemede *et al* (2015) reported 385μg/100 g of β-carotene in fresh okra pods. Patel *et al* (2012) reported maximum of 0.56 mg/g in AOL-8-5 and minimum in AOL-03-1 0.18 mg/g out of 10 different genotypes of okra.

Table 4.4 Variation in chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content in immature pods of wild okra

Genotypes		Chlorophyll a (mg/100 g FW)	Chlorophyll b (mg/100 g FW)	Total Cholorophyll (mg/100 g FW)	Carotenoids (mg/100 g FW)
		Biochemical Parameters			
Abelmoschus moschatus	IC 141056	13.07 ± 0.45^{g}	1.68 ± 0.68^{de}	15.84 ± 1.92^{g}	4.85 ± 0.02^{de}
	IC 140986	13.79 ± 0.73^{fg}	2.73 ± 0.34^{cde}	19.04 ± 0.68^{fg}	5.03 ± 0.05^{cde}
	IC 140985	14.17 ± 0.73^{fg}	3.05 ± 0.29^{cde}	19.26 ± 0.85^{fg}	4.67 ± 0.12^{e}
	IC 470737	17.69 ± 0.25^{e}	4.19 ± 0.34^{cd}	24.42 ± 0.57^{de}	5.02 ± 0.05^{cde}
Abelmoschus mizonagenesis		14.57 ± 0.57^{fg}	2.25 ± 0.45^{cde}	19.21 ± 0.25^{fg}	4.48 ± 0.35^{e}
Abelmoschus tetraphyllus		21.17 ± 0.60^{d}	5.17 ± 1.48^{bc}	27.93 ± 1.35^{d}	5.71 ± 0.28^{cd}
***Abelmoschus angulosus var.grandiflorus* IC 203833**		15.51 ± 0.76^{f}	4.58 ± 0.24^{cd}	22.29 ± 0.80^{ef}	5.78 ± 0.75^{c}
Abelmoschus tuberculatus		30.15 ± 1.50^{c}	8.01 ± 0.76^{ab}	42.46 ± 1.25^{c}	10.11± 0.48^{b}
Abelmoschus manihot	–	38.63 ± 0.23^{a}	8.66 ± 0.85^{a}	52.83 ± 0.83^{a}	13.25±0.23^{a}
	Abelmoschus manihot sp. IC 90339	33.42 ± 0.25^{b}	9.04 ± 2.9^{a}	47.21 ± 2.55^{b}	10.12± 0.13^{b}
Average		20.97	4.94	29.05	6.90
Abelmoschus esculentus	Punjab Padmini	6.68 ± 0.38^{h}	0.69 ± 0.13^{e}	8.35 ± 0.32^{h}	1.86 ± 0.02^{f}
CD (5%)		1.77	2.30	1.37	0.62

Each value is a mean ± SD of three replications

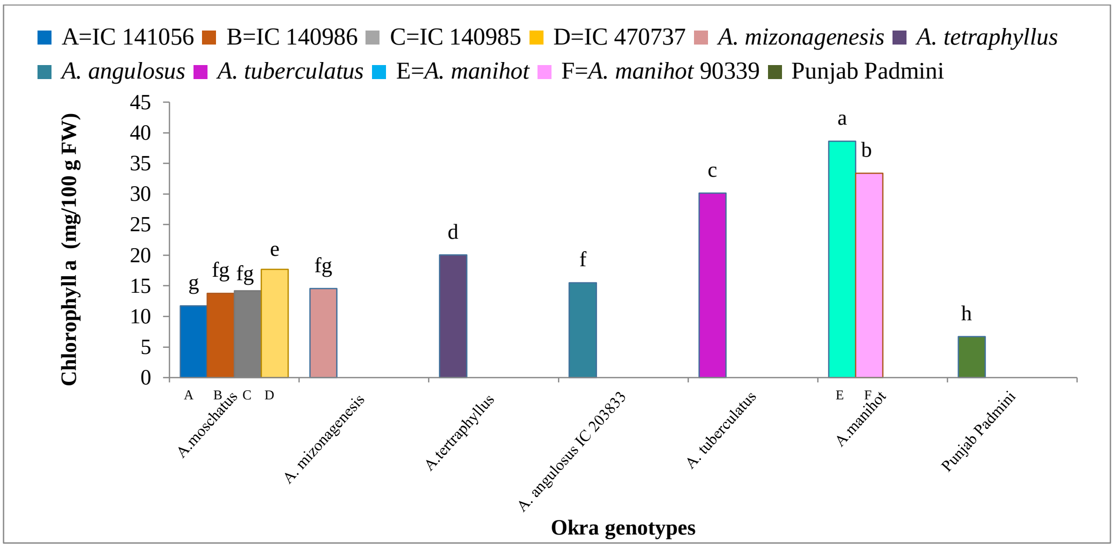

Fig. 4.4(a) Variation in chlorophyll a content in immature pods of wild okra

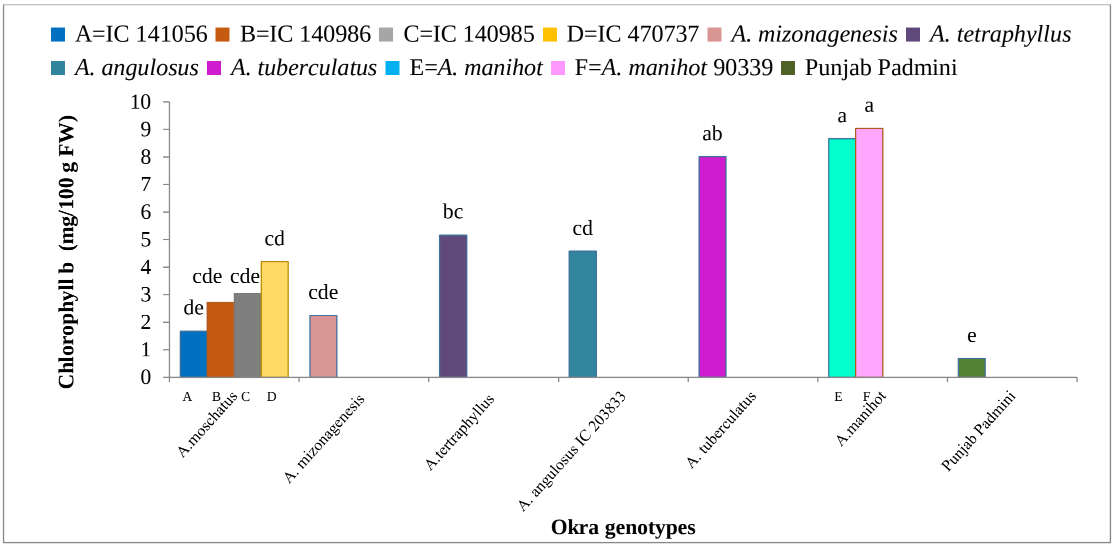

Fig. 4.4(b) Variation in chlorophyll b content in immature pods of wild okra

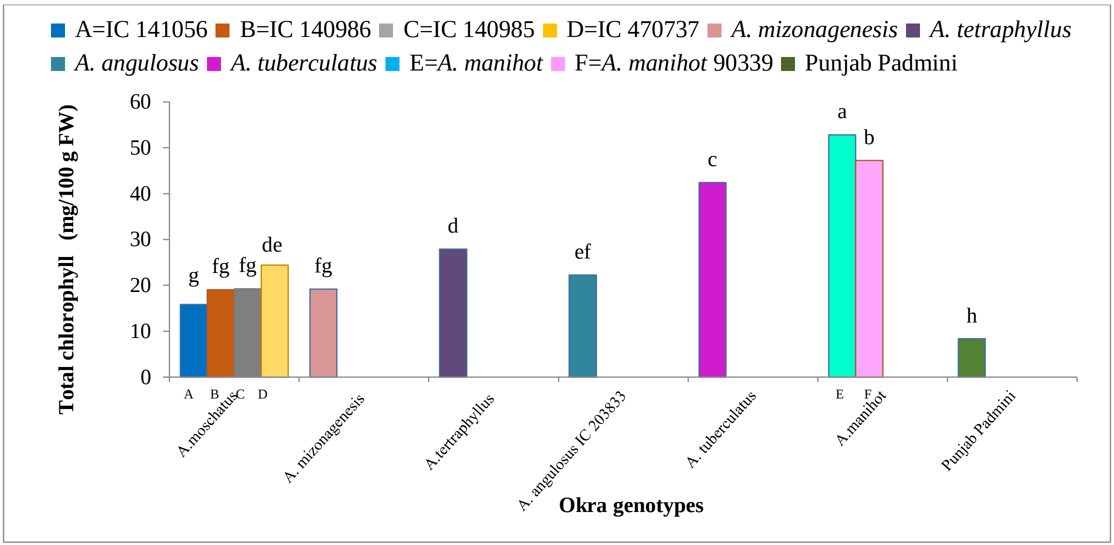

Fig. 4.4(c) Variation in total chlorophyll content in immature pods of wild okra

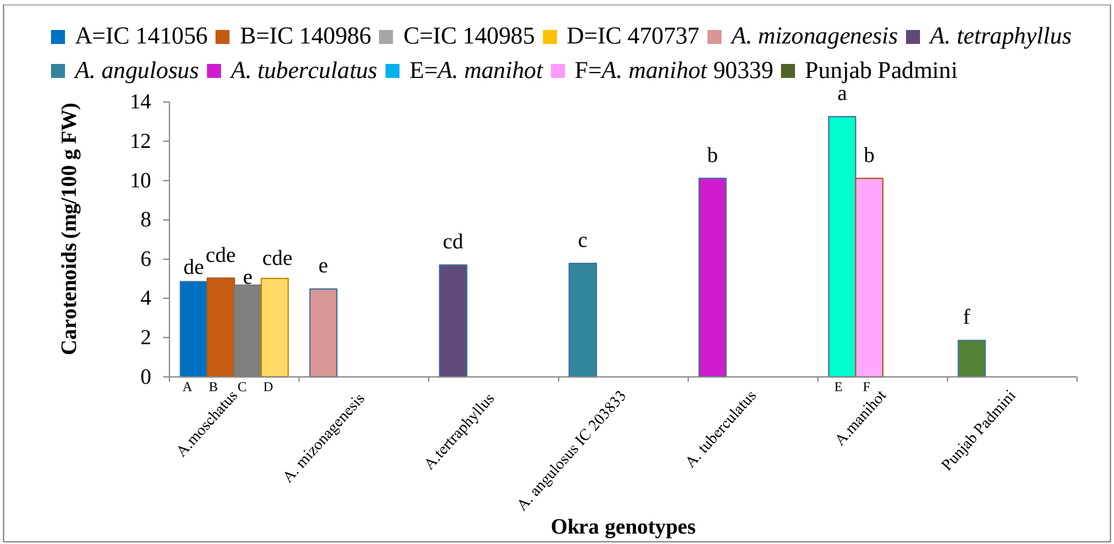

Fig. 4.4(d) Variation in carotenoid content in immature pods of wild okra

Total Phenols

Phenolic compounds are secondary metabolites that may act as antioxidants, attractants (flavonoids and carotenoids), structural polymers (lignin) and signal compounds (salicylic acid and flavonoids). As antioxidants they prevent oxidative damage of biomolecules; such as DNA, proteins, and membrane lipids. (Lin *et al* 2016). Their antioxidant potential is due to chelating of metal ions that are involved in production of free radicals. The phenolics transfer hydrogen atom from hydroxyl group to free radicals and convert them into the stabilised phenoxide radical.

The phenolic content in wild pods varied from 122.95- 412.01 mg/100 g on dry weight basis with average value of 236.52 mg/100 g (Table 4.5). *A. tetraphyllus* registered highest phenolic content followed by *A.manihot* accession 90339, *A.manihot*, and *A.mizonagenesis*. Apart from these the phenolic content in other genotypes was in the range of 130- 196 mg/100 g. Punjab Padmini exhibited phenolic content of 213.61 mg/100 g. The content of O-dihydroxyphenols and flavonols ranged from 4.30- 17.99 mg/100g, and 63.63- 274.49 mg/100g respectively on dry weight basis. The cultivated genotype exhibited 6.08 mg/100g of O-dihydroxyphenol and 134.93 mg/100g DW of Flavonol content. Highest content was recorded in *A. tetraphyllus* (17.99 mg/100g) and *A.manihot sp.* IC 90339 (274.49 mg/100g) respectively. Mohite and Gurav (2019) reported that phenolic content 5.86 mg/g DW was maximum in *A. esculentus* cv. Phule Utkarsha in aqueous extract followed by 4.94 mg/g DW in methanolic extract of *A. ficulneus* and total flavonoid content of 13.93 mg/g DW was highest in *A. esculentus* cv. Phule Utkarsha in aqueous extract followed by 12.07 mg/g DW and 12.04 g/g DW in an ethanolic extract of *A. ficulneus* and *A. manihot* respectively Lalmuanthanga *et al* (2019) reported 117.54 mg GAE/100 g DW of phenolic content in *A. moschatus* roots. Pascal *et al* (2018) registered 28.65 mg GAE /100 mg FW of phenolic content and 71.63 mg EQ /g FW of flavonoids in *A. moschatus.* Sandhi *et al* (2017) reported higher total phenolic content in *A.moschatus* (1.58 mg/g) followed by *A.tetraphyllus* (1.56 mg/g), *A. angulosus* (1.52 mg/g), *A. tuberculatus* (1.46 mg/g) and *A.manihot* (1.43 mg/g). Dhruve *et al* (2015) reported higher phenolic content in wild type (0.68%) and AOL-10-22(0.68) seeds of okra. Xia *et al* (2015) registerted 6.73% of total phenol content in okra immature pods. Sreeshma and Nair (2013) reported total phenolic content at different stages of fruit development process ranged from 0.024mg/ g (1 DAA) to 1.91mg/ g (33 DAA) on DW basis.

Table 4.5 Variation in Total phenols, Vitamin E, Vitamin C, DPPH and FRAP acitivity in immature pods of wild okra

Genotypes		Biochemical Parameters						
		Total phenols (mg/100g DW)	**O -dihydroxy phenols (mg/100g DW)**	**Flavonols (mg/100g DW)**	**Vitamin E (mg/100g DW)**	**Vitamin C (mg/100g FW)**	**DPPH (% inhibition)**	**FRAP (mg AAE/g DW)**
Abelmoschus moschatus	IC 141056	122.95 ± 0.26^{f}	12.03 ± 0.54^{bcd}	91.19 ± 0.83^{f}	28.37 ± 0.77^{cd}	50.18 ± 0.81^{e}	52.78 ± 2.48^{c}	7.93 ± 0.01^{b}
	IC 140986	195.93 ± 0.02^{de}	13.31 ± 1.35^{b}	119.65 ± 1.27^{e}	51.24 ± 0.59^{a}	50.62 ± 0.90^{e}	56.94 ± 0.61^{b}	7.92 ± 0.04^{b}
	IC 140985	133.94 ± 0.15^{ef}	12.36 ± 1.57^{bcd}	89.53 ± 1.44^{g}	27.73 ± 0.96^{cd}	46.26 ± 0.59^{e}	46.45 ± 0.42^{e}	7.96 ± 0.03^{b}
	IC 470737	165.80 ± 0.29^{def}	9.50 ± 0.76^{cde}	141.90 ± 2.91^{c}	37.23 ± 0.88^{b}	41.26 ± 2.78^{f}	54.17 ± 0.01^{bc}	11.19 ± 0.05^{a}
Abelmoschus mizonagenesis		281.77 ± 0.62^{c}	12.94 ± 1.44^{bc}	87.32 ± 0.48^{g}	30.28 ± 1.15^{c}	96.68 ± 1.11^{c}	51.46 ± 0.28^{cd}	7.54 ± 0.07^{c}
Abelmoschus tetraphyllus		412.01 ± 0.27^{a}	17.99 ± 1.54^{a}	146.30 ± 1.10^{c}	26.18 ± 1.06^{de}	111.43 ± 1.42^{b}	39.74 ± 1.96^{f}	11.22 ± 0.01^{a}
***Abelmoschus angulosus var.grandiflorus* IC 203833**		156.82 ± 0.80^{def}	7.81 ± 1.73^{ef}	63.63 ± 1.70^{h}	20.66 ± 1.49^{fg}	41.69 ± 0.80^{f}	45.11 ± 0.05^{e}	5.55 ± 0.04^{d}
Abelmoschus tuberculatus		163.10 ± 0.58^{def}	4.30 ± 1.02^{f}	189.67 ± 1.49^{b}	15.14 ± 0.57^{h}	111.43 ± 0.96^{b}	50.96 ± 0.74^{cd}	4.54 ± 0.02^{f}
	–	338.10 ± 0.08^{bc}	9.37 ± 0.85^{de}	271.33 ± 2.48^{a}	17.87 ± 0.44^{gh}	120.10 ± 1.03^{a}	57.43 ± 0.53^{b}	5.36 ± 0.06^{e}
Abelmoschus manihot	*Abelmoschus manihot sp. IC 90339*	394.78± 0.15^{ab}	8.46 ± 0.41^{e}	274.49 ± 2.49^{a}	18.03 ± 1.41^{gh}	110.10 ± 2.37^{b}	62.90 ± 0.25^{a}	5.66 ± 0.07^{d}
Average		236.52	10.81	147.53	27.27	77.97	51.79	7.49
Abelmoschus esculentus	Punjab Padmini	213.61 ± 0.15^{d}	6.08 ± 1.01^{ef}	134.93 ± 4.16^{d}	23.12± 1.93^{ef}	61.30 ± 2.08^{d}	47.97 ± 2.09^{de}	3.78 ± 0.61^{g}
CD (5%)		1.42	2.09	0.15	1.23	0.16	0.89	0.11

Each value is a mean ± SD of three replications

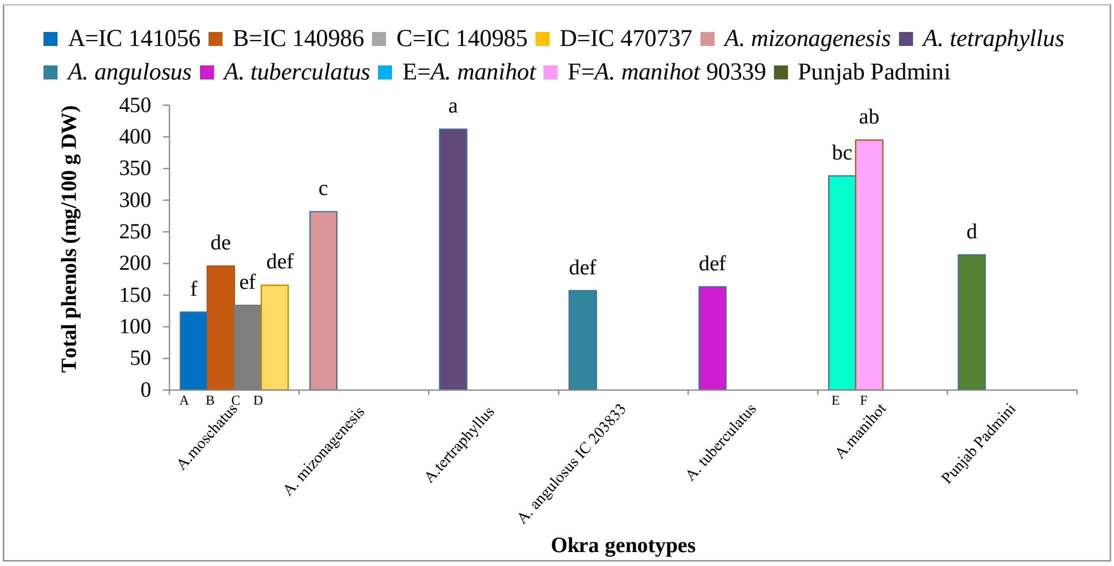

Fig. 4.5(a) Variation in total phenols in immature pods of wild okra

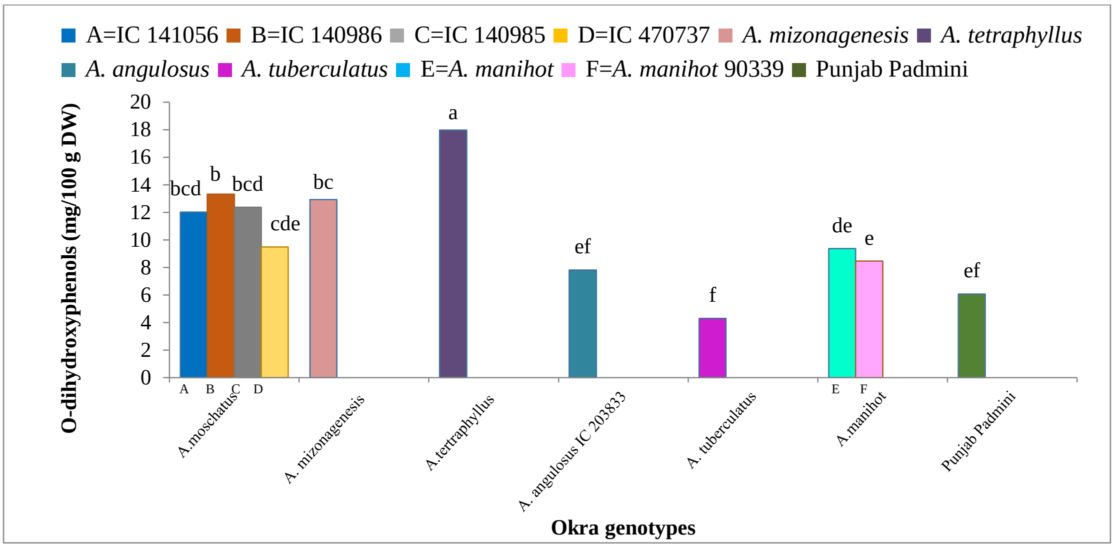

Fig. 4.5(b) Variation in O-dihydroxyphenols in immature pods of wild okra

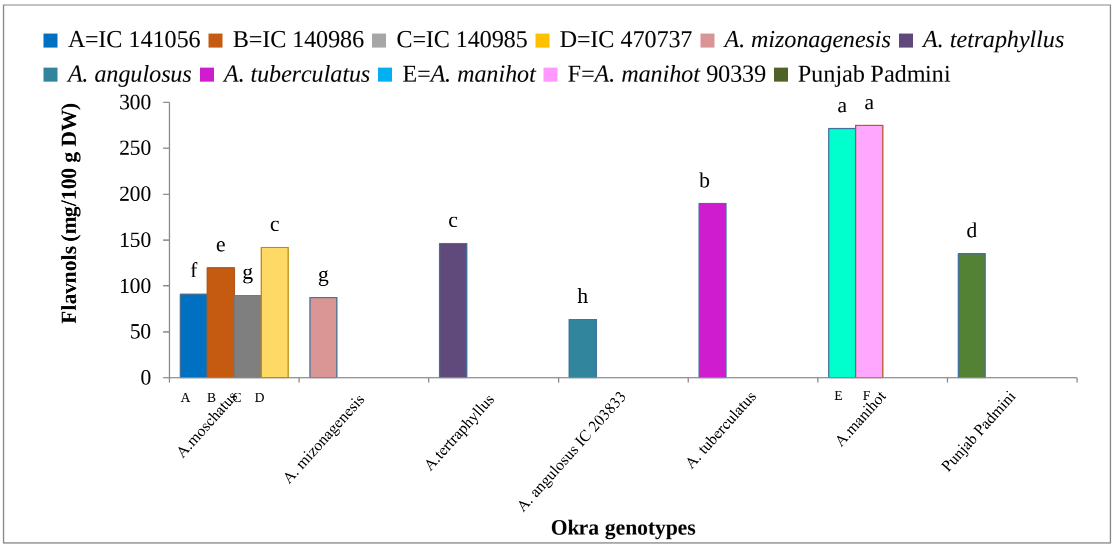

Fig. 4.5(c) Variation in flavanols in immature pods of wild okra

Tocopherol and Ascorbic acid

Vitamin E or tocopherol is naturally occurring fat soluble antioxidant which scavange the hydroperoxyl radicals in lipid milieu and terminate chain reaction. It is the first line of defence against oxidative stress and play essential role in protecting erythrocyte membranes, nervous tissues and PUFAs against oxidative damage (Subasree 2014). Apart from this, Vitamin E also has antiaging properties. In the present study Vitamin E content in wild okra genotypes varied from 15.14- 51.24 mg/100 g on dry weight basis (Table 4.5) with an average of 27.27 mg/100 g while Punjab Padmini recorded 23.12mg/100 g Vit E content. *A.moschatus* accession IC 140986 was richest in Vitamin E content. Petropoulos *et al* (2018) reported total tocopherol content in small and large sized fruit of Mediterranean okra genotypes to be in the range of 0.04- 0.25 mg/100g and 0.050- 0.11 mg/100g respectively on fresh weight basis in relation to harvest stage. Roy *et al* (2014) reported 0.36mg/100 g FW content of vitamin E in okra pods. Kumar *et al* (2013) observed that okra fruit contain 0.27 mg/100g of vitamin E content.

Ascorbic acid is an important water soluble antioxidant vitamin which act as reducing agent and neutralize reactive oxygen species in aqueous phase before initiation of lipid peroxidation. Vitamin C has ability to donate hydrogen atom and forms a relatively stable ascorbyl-free radical. It helps in growth and repair of body tissues, maintain integrity of cells, enhance immunity and also protect lipid membranes and protein from oxidative damage. Okra is a good source of vitamin C. The content of vitamin C ranged from 41.26- 120.10 mg/100g on fresh weight basis with an average 77.97 mg/100g FW. Minimum vitamin C was observed in *A.moschatus* IC 470737 (41.26 mg/100 g) and maximum in *A.manihot* (120.10 mg/100 g). No significant difference was observed between *A.manihot*, *A.tuberculatus* and *A. tetraphyllus*. The cultivated genotype Punjab Padmini registered 61.30 mg/100 g content. Singh *et al* (2014) reported 47 mg/100 g of vitamin c in cultivated okra fruits. Kumar *et al* (2013) registerted the ascorbic acid content of 23 mg/100 g in okra pods. Nair and Fahsa (2013) reported 0.92 mg/ g of vitamin C content in Arka Anamika genotype. Effiong *et al* (2009) registerted that immatutre pods of okra had 203mg/100 g ascorbic acid content.

Total antioxidant activity

The DPPH (1,1-diphenyl-2-picrylhydrazyl) method involve reduction of electron on nitrogen of DPPH by hydrogen atom of antioxidant. While the ferric reducing antioxidant potential assay (FRAP) involve redox reaction in which Ferric iron (Fe^{3+}) is reduced to Ferrous iron (Fe^{2+}) and helps in measuring the reducing power of an antioxidant.

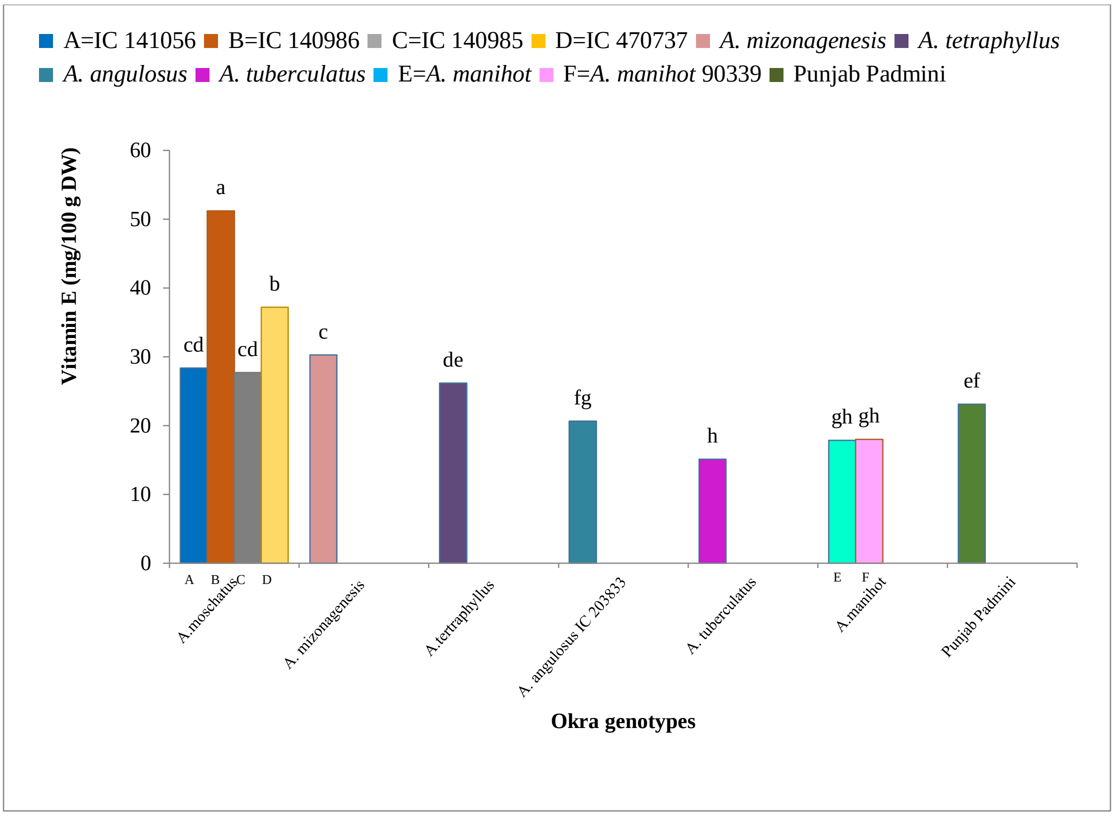

Fig. 4.5(d) Variation in Vitamin E content in immature pods of wild okra

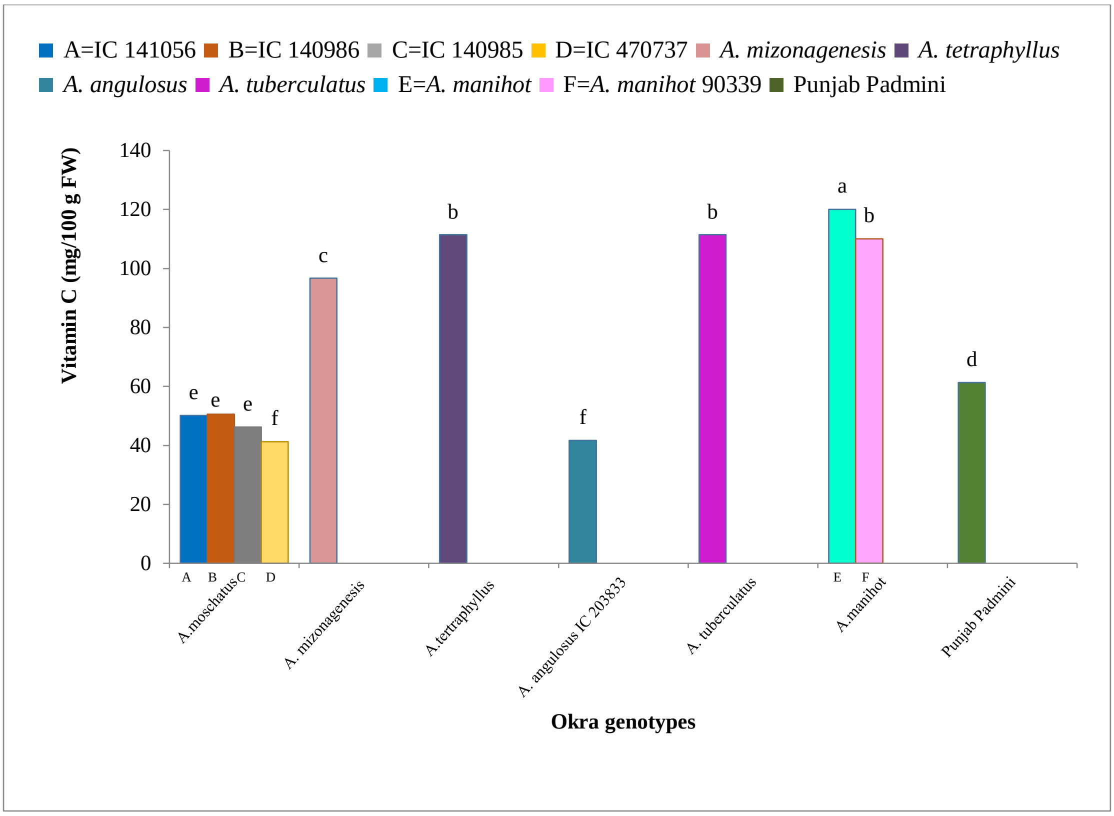

Fig. 4.5(e) Variation in Vitamin C content in immature pods of wild okra

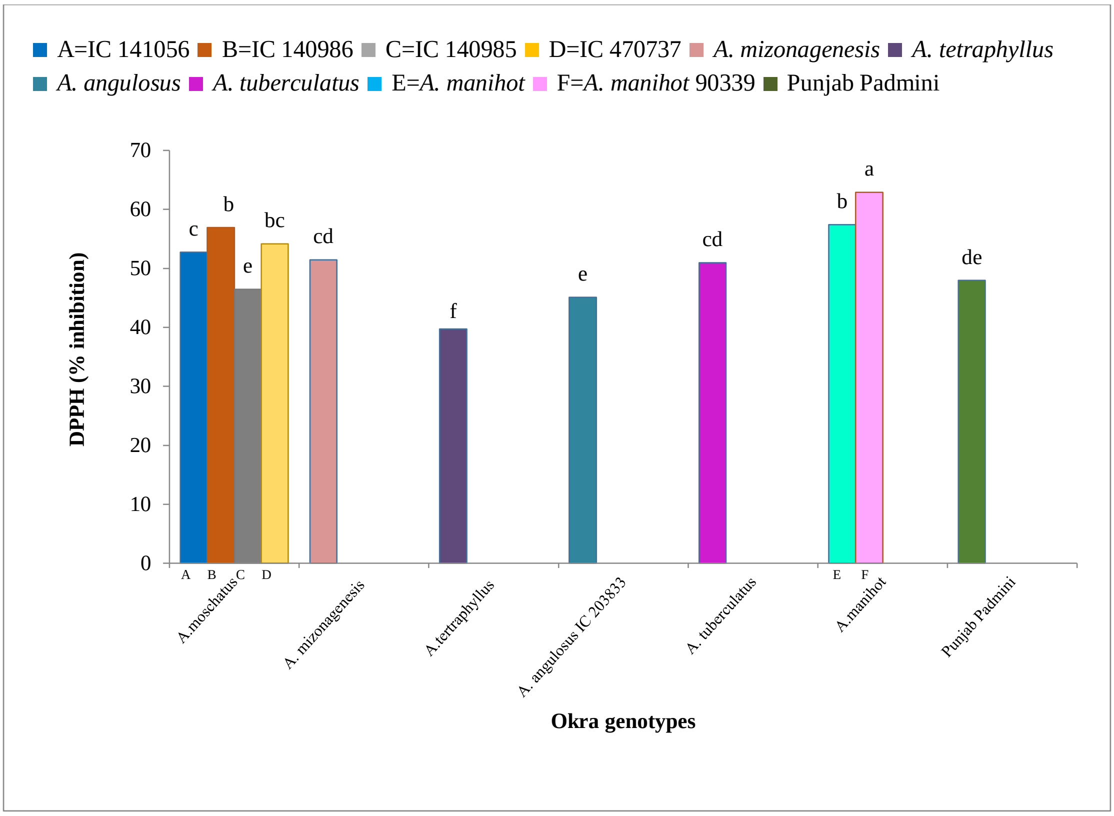

Fig. 4.5(f) Variation in DPPH activity in immature pods of wild okra

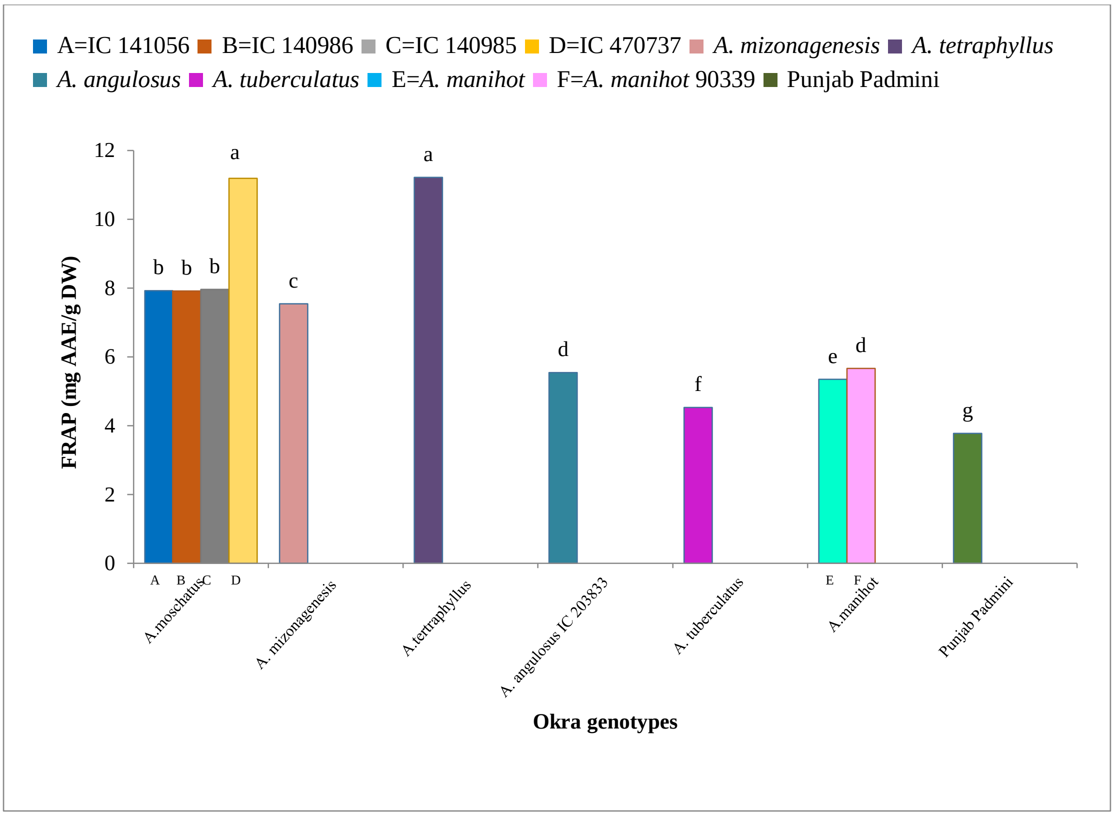

Fig. 4.5(g) Variation in FRAP activity in immature pods of wild okra

DPPH radical scavanging activity in wild okra pods varied from 39.74- 62.90%. The FRAP activity ranged from 4.54- 11.22 mg AAE/g DW (Table 4.5). Among the wild accessions, *A.manihot* accession IC 90339 registered maximum DPPH activity. The DPPH activity of other genotypes ranged between 39- 58%. On the other hand maximum FRAP value was exhibited in *A.tetraphyllus* (11.22 mg AAE/g DW) followed by *A.moschatus* accession IC470737 (11.19 mg AAE/g DW). Punjab Padmini recorded DPPH and FRAP activity of 47.97% and 3.78 mg AAE/g DW respectively. Mohite and Gurav (2019) reported that aqueous extact of *A. ficulneus* had highest content DPPH activity (10.65%) followed by aqueous extract of *A. esculentus* cv. Phule Utkarsha (7.36%) and methanolic extract of *A. manihot* (6.80%). Lalmuanthanga *et al* (2019) registerted DPPH activity of 834.60 mg/ 100 g TE and FRAP activity of 14.00 g TE/ 100 g in roots of *A. moschatus.* Pascal *et al* (2018) reported that DPPH and FRAP activity ranged from 79.81 to 99.26% and 5.72 to 57.91 mmol AAE g^{-1} respectively in *A. caillei* and *A. moschatus.* Petropoulos *et al* (2018) reported FRAP activity ranged from 0.63- 0.91 mg/mL and DPPH activity from 0.66- 1.27 mg/ml in fresh immature fruits of okra in relation to harvest stage. Xia *et al* (2015) registered 1585.48 μmol of TE/g of DPPH scavanging activity and 1429.80 μmol of TE/g of FRAP activity.

4.1.2 Antinutrients

Antinutrients are compounds that reduce availability of nutrients especially vitamins, proteins and minerals by interfering with their absorption. Some antinutrients exert positive effects on health at low concentrations viz. lowering of blood glucose level, cholesterol, triglycerides and also reducing risk of cancer. All the same the beneficial and deleterious effects of antinutrients depend on their chemical structure, concentration, time of exposure and interaction with other dietary components.

Phytic acid is mainly present as K^+, Ca^{2+}, Mg^{2+} phytates and decrease mineral and protein bioavailability. In addition to this, phytate serve as a primary storage of cations, high energy phosphoryl groups and by chelating free iron it also act as an important natural anti-oxidant. Table (4.6) depict the phytate content in wild okra pods. It ranged from 0.65- 2.08% on dry weight basis and its average recorded was 1.49%. Phytate content in cultivated Punjab Padmini was 2.03% that was greater than the average of wild okra. Two genotypes namely *A. tetraphyllus* and *A. tuberculatus* registered lowest phytate content (0.65% and 0.79% respectively) and highest was associated with *A.moschatus* accession IC 470737. Tannins are polyphenolic heat stable compounds with molecular weight in the range of 500 to over 3000 and contain sufficient amount of hydroxyl group that have the ability to precipitate the protein and other macromolecules from aqueous solution. They decrease the protein digestibility either by making the protiens partially unavailable or by inhibiting the digestive enzymes such as trypsin, chymotrypsin, lipase and amylase. It also interfere with iron absorption and at higher concentration cause suppression of microbial enzyme activities. Tannin content in the fruits of wild okra varied from 1.26- 3.30% on dry weight basis. Maximum tannin content was was observed in *A.tetraphyllus* (3.30%) followed by *A.moschatus* accession IC 140986 (2.66%), IC 141056 (2.08%), IC 140985(2.07%). The mean of wild genotypes was observed to be more than Punjab Padmini (1.08%). Oxalate binds to calcium to form calcium oxalate which is insoluble. This insoluble calcium oxalate salt results in formation of kidney stones. Moreover, when oxalic acid binds with essential nutrients it prevents absorption and utilisation of these nutrients and thus render them inaccessible to the body.

Table 4.6 Variation in phytate, tannins, oxalate and saponin content in immature pods of wild okra

Genotypes		Biochemical Parameters			
		Phytate (g/100g DW)	**Tanins (g/100g DW)**	**Oxalate (g/100g DW)**	**Saponin (g/100g DW)**
Abelmoschus moschatus	IC 141056	1.90 ± 0.02[b]	2.08 ± 0.03[c]	0.43 ± 0.08[e]	0.39 ± 0.02[g]
	IC 140986	1.91 ± 0.22[b]	2.66 ± 0.08[b]	0.39 ± 0.25[g]	0.56 ± 0.07[f]
	IC 140985	1.92 ± 0.08[b]	2.07 ± 0.25[c]	0.44 ± 0.09[d]	0.73 ± 0.08[d]
	IC 470737	2.08 ± 0.36[a]	1.65 ± 0.15[e]	0.37 ± 0.19[h]	0.79 ± 0.06[c]
Abelmoschus mizonagenesis		1.92 ± 0.05[b]	1.77 ± 0.09[d]	0.26 ± 0.25[j]	0.63 ± 0.16[e]
Abelmoschus tetraphyllus		0.65 ± 0.07[g]	3.30 ± 0.19[a]	0.23 ± 0.20[k]	1.04 ± 0.23[b]
***Abelmoschus angulosus var.grandiflorus* IC 203833**		1.01 ± 0.06[e]	1.75 ± 0.12[d]	0.63 ± 0.40[a]	0.39 ± 0.07[g]
Abelmoschus tuberculatus		0.79 ± 0.13[f]	1.59 ± 0.02[f]	0.49 ± 0.46[c]	0.30 ± 0.13[h]
Abelmoschus manihot	–	1.62 ± 0.19[c]	1.26 ± 0.10[g]	0.52 ± 0.37[b]	0.19 ± 0.07[i]
	Abelmoschus manihot sp. IC 90339	1.16 ± 0.09[d]	1.28 ± 0.11[g]	0.42 ± 0.40[f]	0.32 ± 0.10[h]
Average		1.49	1.94	0.42	0.53
Abelmoschus esculentus	Punjab Padmini	2.03 ± 0.30[a]	1.08 ± 0.14[h]	0.26 ± 0.17[i]	1.44 ± 0.24[a]
CD (5%)		0.68	0.59	0.28	0.78

Each value is a mean ± SD of three replications

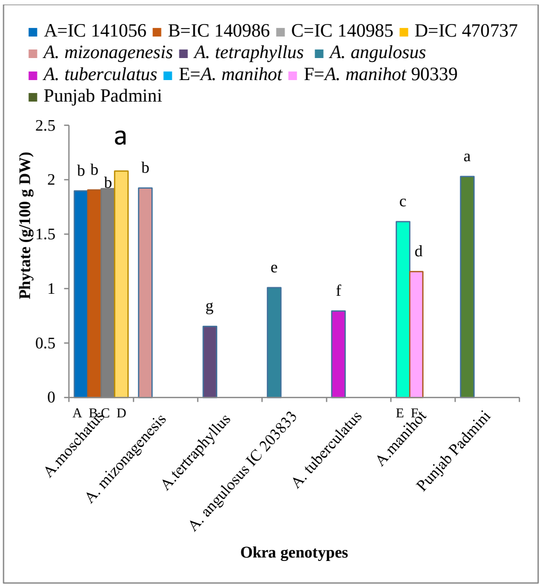

Fig. 4.6(a) Phytate

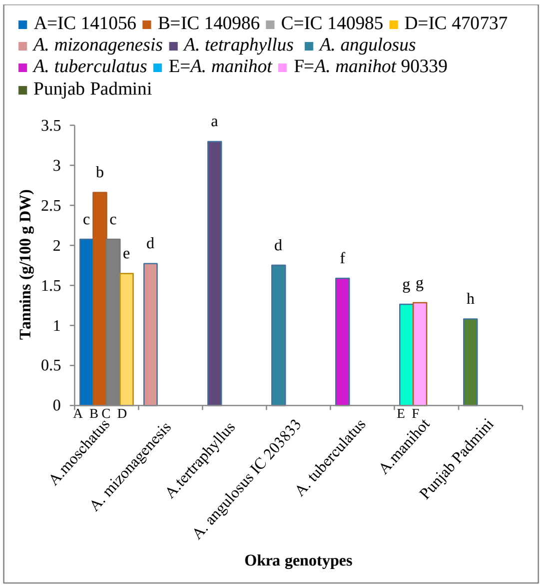

Fig. 4.6(b) Tannins

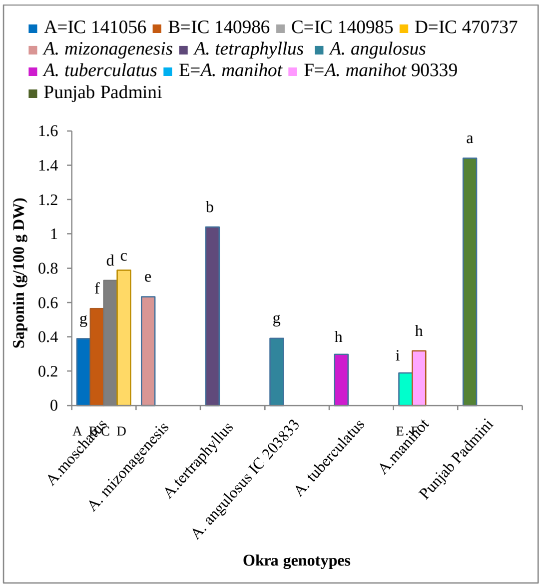

Fig. 4.6(d) Saponins

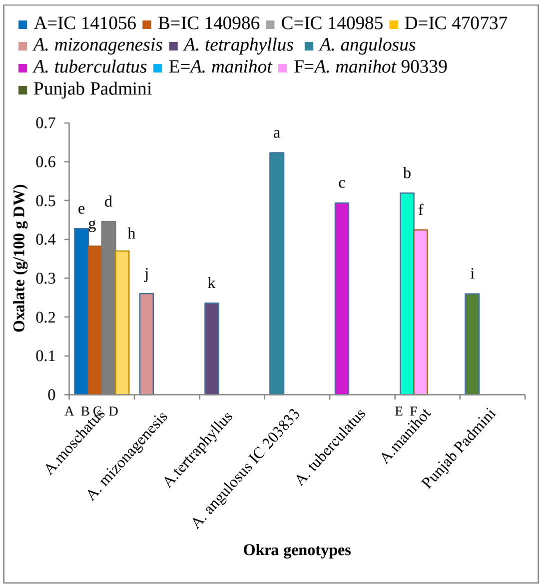

Fig. 4.6(c) Oxalate

Fig. 4.6 Variation in phytate (a), tannins (b), oxalate (c) and saponin (d) content in immature pods of wild okra

In present study the wild genotypes registered oxalate content in the range of 0.23-0.63% on dry weight basis. *A. tetraphyllus* and *A.mizonagenesis* registered low oxalate content and *Abelmoschus angulosus var.grandiflorus* IC 203833 depicted high content. Cultivated variety Punjab Padmini harboured lower oxalate content of 0.26%. Saponins are naturally occurring non-volatile and surface active compound that are widely distributed in plant. They are structurally diverse and also known as triterpene and steroid glycosides. The structural complexity of saponins endows upon them diverse properties, like sweetness, bitterness, pharmacological and medicinal properties, haemolytic properties, immunostimulatory, hypocholesterolemic, anticarcinogenic properties as well as antimicrobial activities (Marrelli *et al* 2016). They reduce the bioavailability of essential nutrients to body and decrease the protein digestibility by inhibiting various digestive enzymes such as chymotrypsin and trypsin. Fig (4.6) depicted that the saponin content in immature pods of wild okra varied from 0.19- 1.04% on dry weight basis with average of 0.53%. Punjab Padmini registered content of 1.44%. Moreover, none of the wild genotype had the saponin content greater than Punjab Padmini except *A.tetraphyllus* which was at par with it. In wild species, minimum content was harboured by *A. manihot* (0.19%). Yora and Sobir (2018) reported that thirteen genotypes of okra had positive amount of saponin and tannin content in their immature fruits. Ehilé *et al* (2018) reported the phytate and oxalate content in *A.tuberculatus* was 1070 mg/100g DW and 382 mg/100g DW respectively. Gemede *et al* (2018) also registered the range of 0.39 to 0.46, 0.71 to 3.78, and 0.74 to 0.75 mg/100 g of phytate, tannin and oxalate content respectively in seeds of eight accessions of okra. Petropoulos *et al* (2018) reported the oxalic acid content of 0.050-0.187% and 0.112-0.217% in small and large sized fruits of okra. Adamma *et al* (2014) also reported the immature fruits of *A. esculentus* for phytate, saponin and oxalate composition with value of 0.66 mg/g, 0.28 mg/g and 0.85 mg/g respectively. Pascal *et al* (2018) reported the content of condensed tannins to be very low in okra genotypes namely *A. moschatus* and *A. caillie.* It ranged from 0.91- 13.60 mg/g FW. Firmin *et al* (2018) evaluated 1652.78 mg TAE/ 100g DW of the oxalate content in okra pods. Sandhi *et al* (2017) reported that tannin content in wild okra genotypes (*A. moschatus, A.tetraphyllus* and *A. angulosus*) ranged from 26.12–31.48 mg/g. Gemede *et al* (2015) registerted the range of 4.93–9.90, 0.83–0.87 and 0.04–0.53 (mg/100 g) for tannin, phytate and oxalate contents in pods of okra accessions respectively. Nair and Fahsa (2013) exhibited the presence of saponins in mucilage of some species okra (*A.esculentus, A.manihot*, *A.msochatus, A.angulosus* and *A.caillie*). Ndlovu and Afolayan (2008) reported 11.71% of phytate content in the leaves of wild *C. olitorious.*

4.2 Biochemical characterisation of mature seeds

Okra seeds are rich source of protein and oil. Okra seed flour can be used to fortify cereal flour to improve its quality in terms of amino acids and also used to supplement corn flour to make better quality dough. In addition to this, mature okra seeds are used to prepare okra cheese and other bakery products. It is also considered to be a good source of crude fiber mainly present in seed coat.

4.2.1 Proteins

The protein content in seeds of okra varies from 15-26% (Chanchal *et al* 2018). The biological value of proteins is characterized by the content of amino acid, its digestibility by humans. It is similar to soybean seed protein in amino acid composition but better in protein efficiency ratio (PER) than soybean seed protein (Hassan and Ali *et al* 2015). Okra is called "a perfect villager's vegetable" due to good dietary fiber content and presence of tryptophan and lysine in seed protein (Kumar *et al* 2010). The data in Table (4.7) represented the crude protein and total soluble protein content in different okra species. The crude protein content in the seeds of wild okra varied from 19.35- 27.09% on dry weight basis and recorded the mean value to be 21.50%. The total soluble protein ranged from 9.83- 19%. The mean value registered was 12.71%. Among the wild accessions, *A.tuberculatus* had the maximum content of both the entities with values of 27.09% and 19% respectively. The lowest content was recorded in *A. mizonagenesis* (19.35% and 9.38% respectively). With respect to both the parameters (crude protein and total soluble protein), all the wild genotypes of okra seeds were at par except for the seeds of *A. tuberculatus*. The cultivated genotype depicted crude protein value of 21.71% and total soluble protein value of 13.16% in its seeds. Dubey *et al* (2013) reported seed protein content in two ambrette germplasm of *A.moschatus* with value of 19.16 mg/g and 14.23 mg/g respectively. Gemede *et al* (2018) reported 22.51- 38.09 g/100 g DW of

Table 4.7 Variation in crude protein and total soluble protein content in mature seeds of wild okra

Genotypes		Biochemical Parameters	
		Crude protein (% DW)	**Total soluble proteins (% DW)**
Abelmoschus moschatus	IC 141056	19.72 ± 0.12^{f}	11.48 ± 0.05^{h}
	IC 140986	19.39 ± 0.03^{g}	12.27 ± 0.09^{f}
	IC 140985	21.79 ± 0.06^{d}	12.63 ± 0.04^{e}
	IC 470737	22.58 ± 0.03^{c}	12.70 ± 0.25^{de}
Abelmoschus mizonagenesis		19.35 ± 0.02^{g}	9.83 ± 0.04^{i}
Abelmoschus tetraphyllus		23.17 ± 0.03^{b}	11.58 ± 0.05^{h}
***Abelmoschus angulosus var.grandiflorus* IC 203833**		19.51 ± 0.19^{fg}	11.93 ± 0.15^{g}
Abelmoschus tuberculatus		27.09 ± 0.03^{a}	19 ± 0.11^{a}
Abelmoschus manihot	–	20.48 ± 0.04^{e}	12.88 ± 0.07^{c}
	Abelmoschus manihot sp. IC 90339	21.87 ± 0.10^{d}	12.86 ± 0.06^{cd}
Average		21.50	12.71
Abelmoschus esculentus	Punjab Padmini	21.71 ± 0.19^{d}	13.16 ± 0.20^{b}
CD (5%)		0.12	0.73

Each value is a mean ± SD of three replications

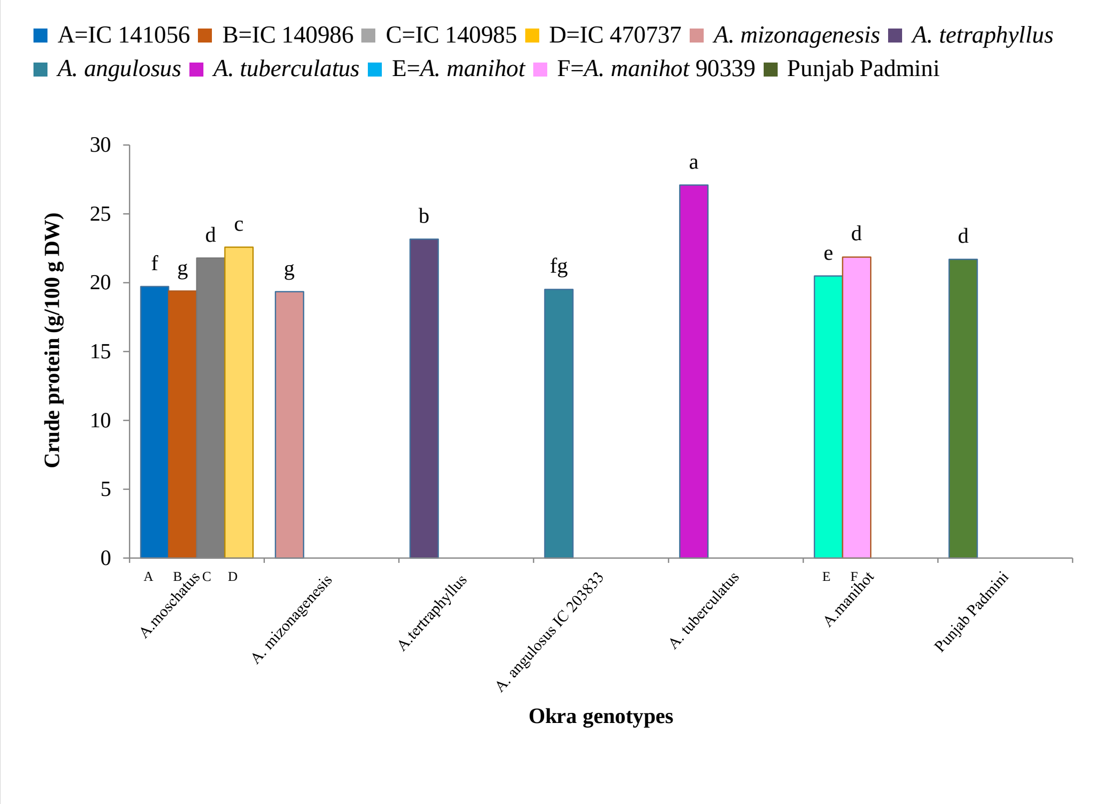

Fig. 4.7(a) Variation in crude protein content in mature seeds of wild okra

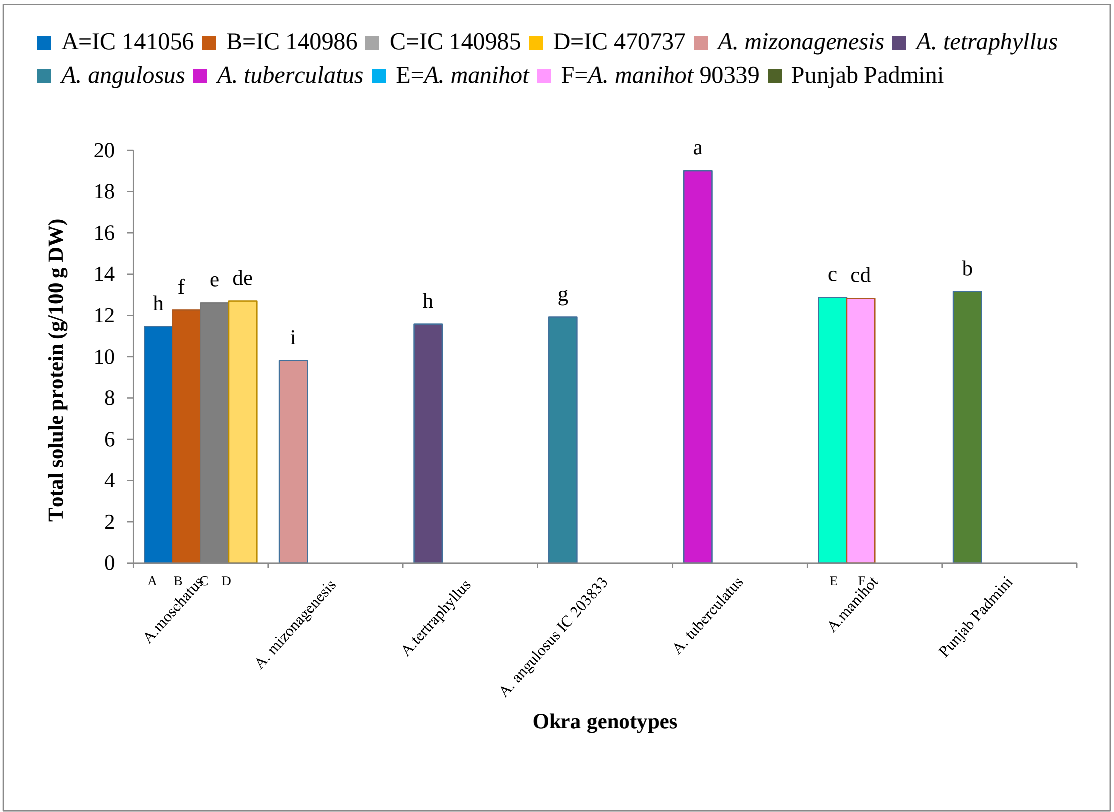

Fig. 4.7(b) Variation in total soluble proteins in mature seeds of wild okra

crude protein in eight accessions of okra seeds. Liu Jie *et al* (2017) registered the total soluble protein content of 10.36%-14.51% in seeds of *A. manihot.* Petropoulus *et al* (2017) reported the 378-407 g kg^{-1} DW of protein content in okra seeds. Dhruve *et al* (2015) recorded the minimum protein content in AOL-13-88 and maximum in GAO-5 with value of 14.05% and 18.96% respectively in okra seeds. Ndangui *et al* (2010) also reported crude protein content of 24.85% in okra seeds.

4.2.2 Oil content and Fatty acid profile

Okra seeds contain good amount of oil and has been used for oil production on small scale. In almost all types of foods, lipid and fatty acid composition contribute to physical, nutritional and sensory quality. The okra seed oil mainly consist of unsaturated essential fatty acids such as linoleic acid and oleic acid (Abdulrahaman *et al* 2015). Its yield is comparable to most of oil seed crops except soyabean and palm oil (Sanjeet *et al* 2010). Moreover, okra seed oil has potential as edible oil and has hypocholesterolemic effect (Sanjeet *et al* 2010).

Fig (4.8) depicted that total oil content in wild okra seeds ranged from 10.68-27.61% DW with a mean value of 15.56%. The cultivated genotype Punjab Padmini registered 8.83% of oil. *A. mizonagenesis* harboured maximum oil content i.e 27.61% and minimum content was observed in *A. moschatus* accession IC 141056 (10.68%). Three accessions of *A. moschatus* (IC 141056, IC 140986, IC 140985) and *A angulosus var. grandiflorus* IC 203833 are at par with each other with respect to oil content. However the fourth *A. moschatus* accession IC 470737 is at par with *A. tuberculatus* and *A.manihot* for oil content. Regarding fatty acids palmitic acid, stearic acid, oleic acid and Linoleic acid varied from 21.13- 26.25%, 2.28- 4.56%, 23.51- 34.47% and 31.49- 48.67% on dry weight basis with mean value of 4.16% , 3.10%, 28.14% and 43.12% respectively. Highest content of palmitic, stearic, oleic and linoleic acid was found in *A. mizonagenesis* (25.93%), *A. moschatus* accession IC 141056 (4.56%), *A. moschatus* accession IC 140986 (34.47%), *A. moschatus* accession IC 470737 (47.78%) respectively. It was observed that cultivated Punjab Padmini had higher content of palmitic (30.58%), stearic (3.77%) and oleic (28.84 %) than mean value of wild genotypes but with respect to linoleic acid mean value of wild genoptyes had higher content 43.12% than Punjab Padmini (36.68%).

Dhruve *et al* (2015) reported that seed oil content ranged from 10.94 to 15.70% in ten okra genotypes. Maximum oil content was found in AOL-10-22 (15.70%) while minimum in wild type (10.94%). Liu Jie *et al* (2017) registered total fatty acid and unsaturated fatty acid in seeds of *A.manihot* varied from 55.47- 102.17mg/g and 78.01- 79.40% respectively. Petropoulos *et al* (2017) reported that maximum fatty acid content was found in linoleic acid followed by palmitic and oleic acid with range 40.69-48.01%, 27.1- 28.50% , 16.98-25.58% respectively in seeds of four Greek okra genotypes. Hassan and Ali (2015) registerted the fat content of 16.06-23.99% in okra seed flour and 2.65 to 3.68% in defatted okra seed flour.

Table 4.8 Variation in oil content and fatty acid composition in mature seeds of wild okra

Genotypes		Biochemical Parameters				
		Total oil content in seed (% DW)	**Palmitic acid (%)**	**Stearic acid (%)**	**Oelic acid (%)**	**Linoleic acid (%)**
Abelmoschus moschatus	IC 141056	10.68 ± 0.25^{f}	21.13 ± 0.10^{g}	4.56 ± 0.07^{a}	33.42 ± 0.09^{c}	41.22 ± 0.03^{f}
	IC 140986	11.17 ± 1.04^{ef}	22.57 ± 0.10^{f}	3.58 ± 0.06^{bc}	34.47 ± 0.08^{a}	39.63 ± 0.04^{g}
	IC 140985	12.28 ± 0.52^{e}	22.85 ± 0.05^{f}	3.30 ± 0.06^{d}	34.15 ± 0.04^{b}	39.29 ± 0.05^{h}
	IC 470737	16.36 ± 0.60^{c}	23.35 ± 0.27^{e}	3.44 ± 0.12^{cd}	25.10 ± 0.08^{h}	48.67 ± 0.05^{a}
Abelmoschus mizonagenesis		27.61 ± 0.09^{a}	25.93 ± 0.07^{b}	2.65 ± 0.08^{ef}	23.51 ± 0.14^{j}	48.18 ± 0.05^{b}
Abelmoschus tetraphyllus		14.26 ± 0.18^{d}	24.25 ± 0.07^{d}	2.84 ± 0.06^{e}	24.42 ± 0.07^{i}	31.49 ± 0.04^{j}
***Abelmoschus angulosus var.grandiflorus* IC 203833**		11.23 ± 0.06^{ef}	24.55 ± 0.09^{cd}	2.50 ± 0.08^{fg}	25.54 ± 0.12^{g}	47.78 ± 0.06^{c}
Abelmoschus tuberculatus		16.54 ± 0.14^{c}	26.25 ± 0.10^{b}	3.22 ± 0.07^{d}	27.17 ± 0.04^{e}	45.39 ± 0.06^{d}
Abelmoschus manihot	–	17.04 ± 0.18^{c}	25.87 ± 0.08^{b}	2.60 ± 0.17^{ef}	26.52 ± 0.03^{f}	45.29 ± 0.06^{d}
	Abelmoschus manihot sp. IC 90339	18.54 ± 0.15^{b}	24.87 ± 0.06^{c}	2.28 ± 0.07^{g}	27.06 ± 0.04^{e}	$44.29 \pm 0 .07^{e}$
Average		15.56	24.16	3.10	28.14	43.12
Abelmoschus esculentus	Punjab Padmini	8.83 ± 0.30^{g}	30.58 ± 0.31^{a}	3.77 ± 0.09^{b}	28.84 ± 0.06^{d}	36.68 ± 0.09^{i}
CD (5%)		0.64	0.15	0.25	0.87	0.55

Each value is a mean ± SD of three replications

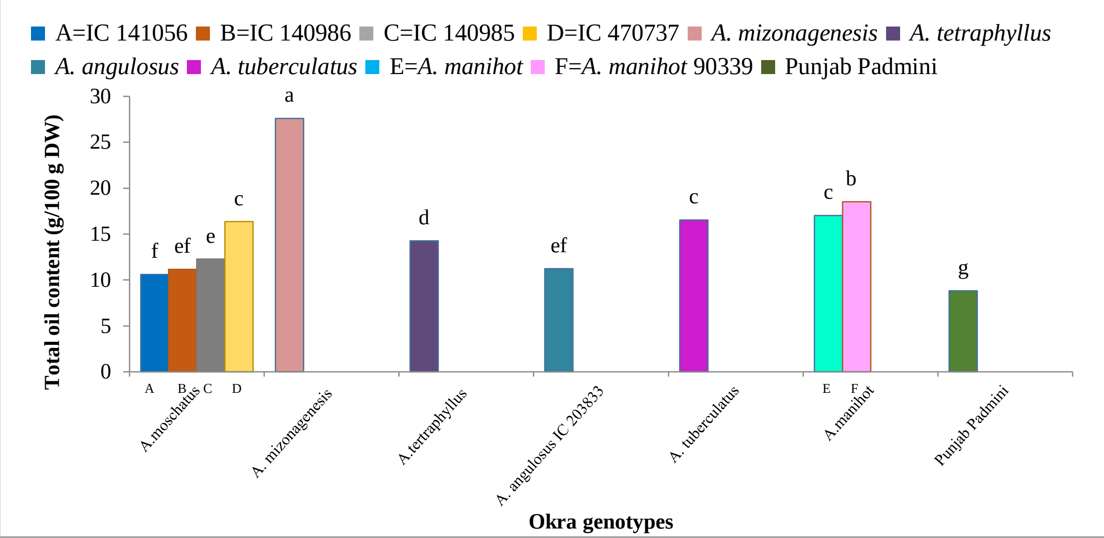

Fig. 4.8(a) Variation in total oil content in mature seeds of wild okra

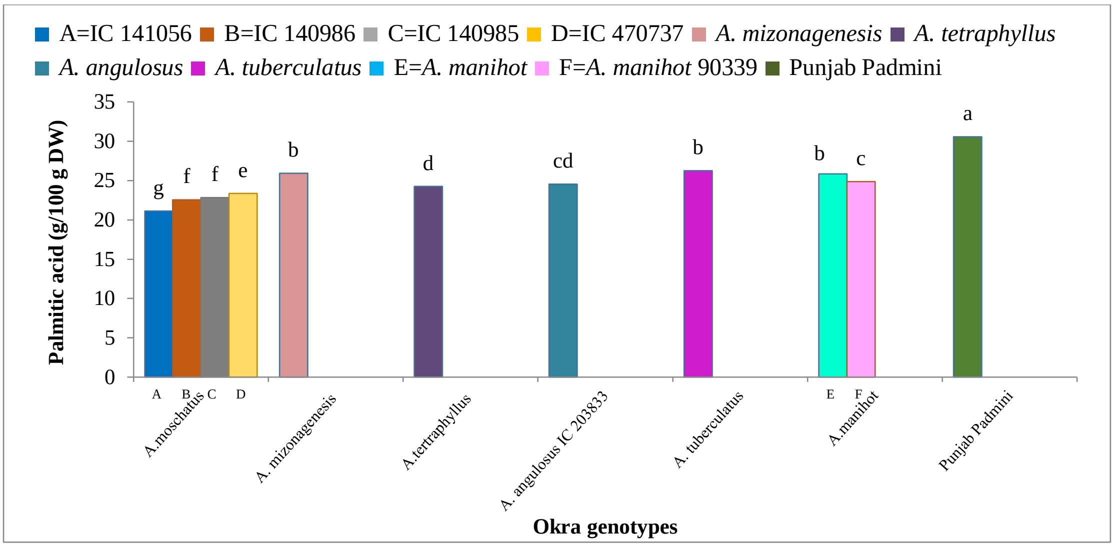

Fig. 4.8(b) Variation in palmitic acid content in mature seeds of wild okra

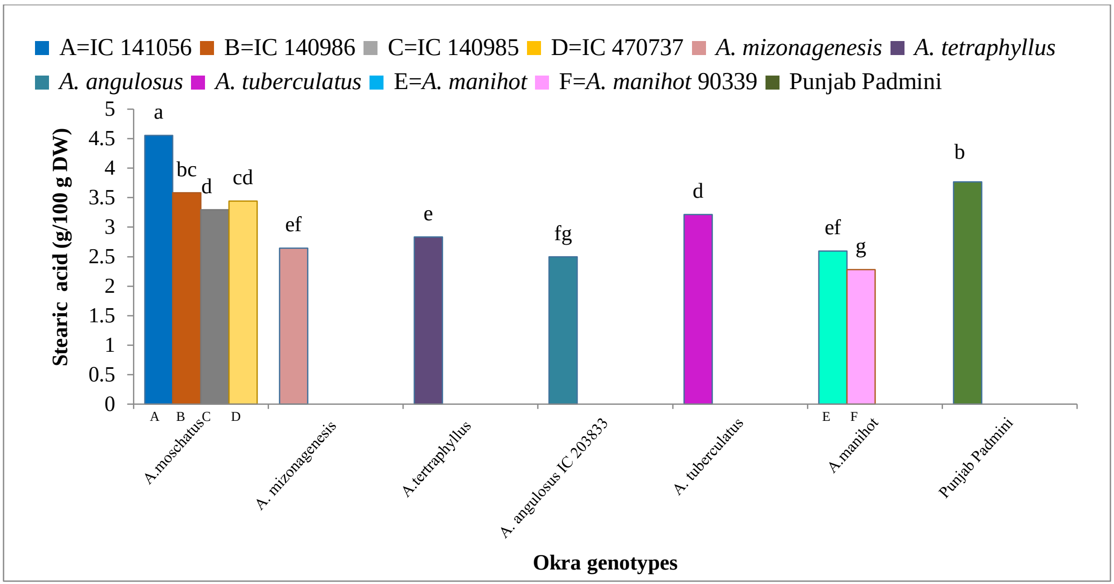

Fig. 4.8(c) Variation in stearic acid content in mature seeds of wild okra

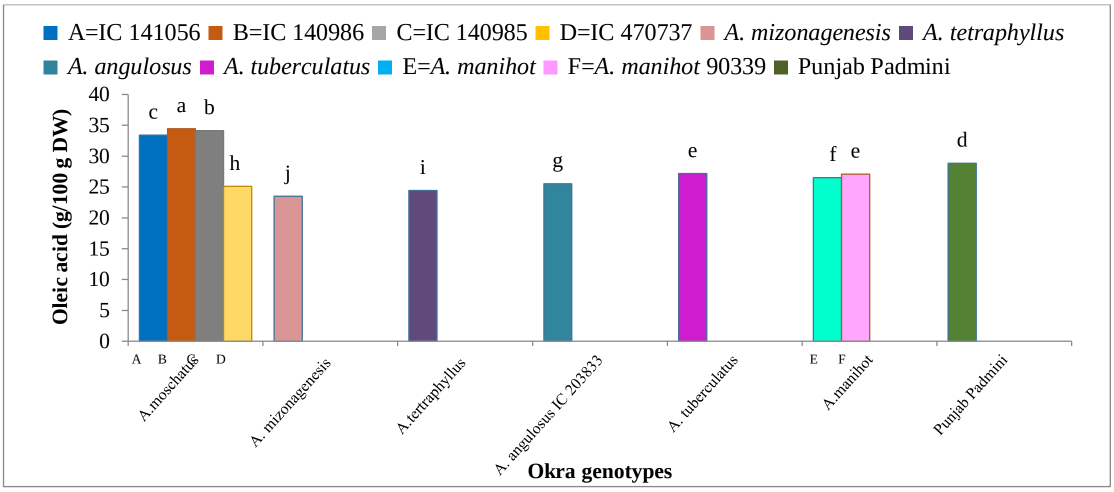

Fig. 4.8(d) Variation in oleic acid content in mature seeds of wild okra

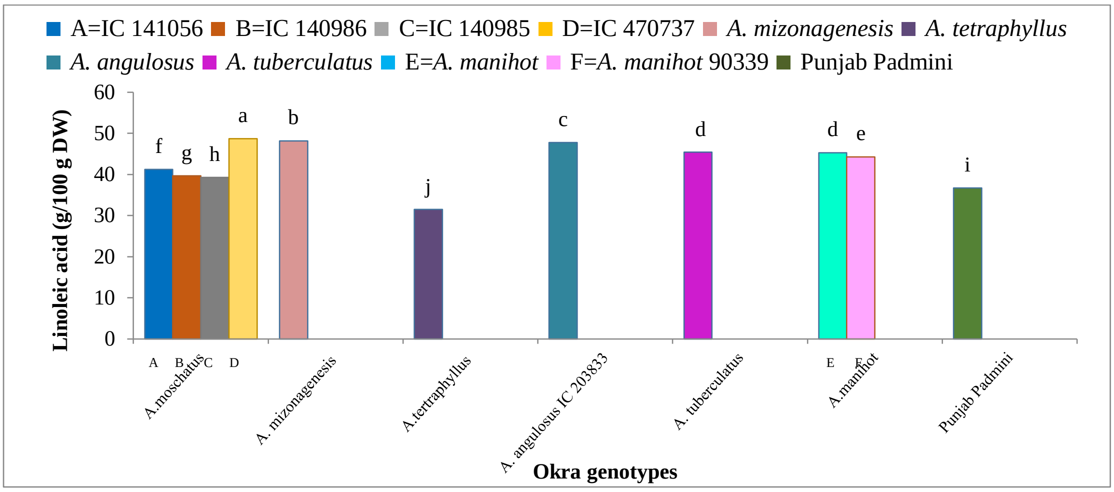

Fig. 4.8(e) Variation in linoleic acid content in mature seeds of wild okra

Dong *et al* (2014) reported oil content in mature seeds of okra ranged of 8.25- 16.88%. Jarret *et al* (2011) reported that oil content in seeds of different accessions *A. esculentus*, *A. caillei*, *A. manihot*, *A. ficulneus*, *A. tuberculatus* and *A. moschatus* ranged from 12.36- 21.56%, 2.51- 13.61%, 16.1- 22.0%, 6.62- 16.7%, 10.8- 23.2% and 10.3- 19.8% respectively. It also examined the composition of fatty acid which ranged from 23.6- 50.65% for linoleic acid in *A. esculentus.* However the average linoleic acid content was maximum in *A. ficulneus* and *A. tuberculatus.*

4.2.3 Carotenoids and Tocopherols

Apart from high protein and oil content, okra seed also contain higher antioxidant activity**.** The mature seeds are rich in antioxidant phenolic compounds such as carotenoids and Vitamin E. Tocopherols prevent the oxidation of low-density lipoprotein (LDL) which are causative agent of atherosclerosis and heart attack. It also plays an important role in protecting vitamin A and C from oxidation (Kanani *et al* 2019).

Seeds of wild okra registered carotenoid content in the range of 1.27- 7.11 mg/100 g on dry weight basis (Table 4.9). Eight genotypes were at par with no significant difference for carotenoids. The cultivated Punjab Padmini depicted 3.38% of carotenoids. Maximum amount of carotenoid content was found in *Abelmoschus manihot* (7.11 mg/100g) and minimum in *Abelmoschus angulosus var.grandiflorus* IC 203833 (1.27 mg/100g). Kanani *et al* (2019) reported 2.22- 2.81mg/kg of carotenoid content in seed oil of three Iraqi okra. Furthermore the Vitamin E content in seeds ranged from 26.08- 107.63 mg/100g on dry weight basis with average value of 57.29 mg/100g (Table 4.9). Among all the wild accessions, *A.moschatus* accession IC 140986 had maximum value of tocopherol content ranged 107.63mg/100g and minimum was found in *A.manihot sp. IC 90339* (26.08 mg/100g).Eight wild genotypes namely *A.moschatus* accessions (IC 141056, IC140985, IC 470737), *A. mizonagenesis*, *A.tetraphyllus*, *A. angulosus* I C 203833, *A.tuberculatus, A. manihot* had medium value of vitamin E content ranged from 40.62- 87.99 mg/100g. It was also observed that Punjab Padmini (40.52 mg/100g) had lowest content of vitamin E than the average value of wild genotypes. Anwar *et al* (2011) reported total tocopherol content in seed

Table 4.9 Variation in carotenoids and vitamin E content in mature seeds of wild okra

Genotypes		**Biochemical Parameters**	
		Carotenoids (mg/100g DW)	**Vitamin E seed (mg/100g DW)**
Abelmoschus moschatus	IC 141056	2.79 ± 0.10^{f}	87.99 ± 1.72^{b}
	IC 140986	3.85 ± 0.02^{d}	107.63 ± 2.19^{a}
	IC 140985	3.96 ± 0.09^{d}	60.67 ± 1.00^{d}
	IC 470737	3.99 ± 0.07^{d}	41.66 ± 2.03^{f}
Abelmoschus mizonagenesis		5.66 ± 0.06^{b}	66.70 ± 2.77^{c}
Abelmoschus tetraphyllus		3.87 ± 0.03^{d}	48.62 ± 1.74^{e}
***Abelmoschus angulosus var.grandiflorus* IC 203833**		1.27 ± 0.10^{g}	40.62 ± 0.65^{f}
Abelmoschus tuberculatus		3.51 ± 0.10^{e}	41.66 ± 1.72^{f}
Abelmoschus manihot	–	7.71 ± 0.07^{a}	51.32 ± 1.98^{e}
	Abelmoschus manihot sp. IC 90339	4.46 ± 0.06^{c}	26.08 ± 0.65^{g}
Average		4.11	57.29
Abelmoschus esculentus	Punjab Padmini	3.38 ± 0.08^{e}	40.52 ± 0.94^{f}
CD (5%)		0.22	0.19

Each value is a mean ± SD of three replications

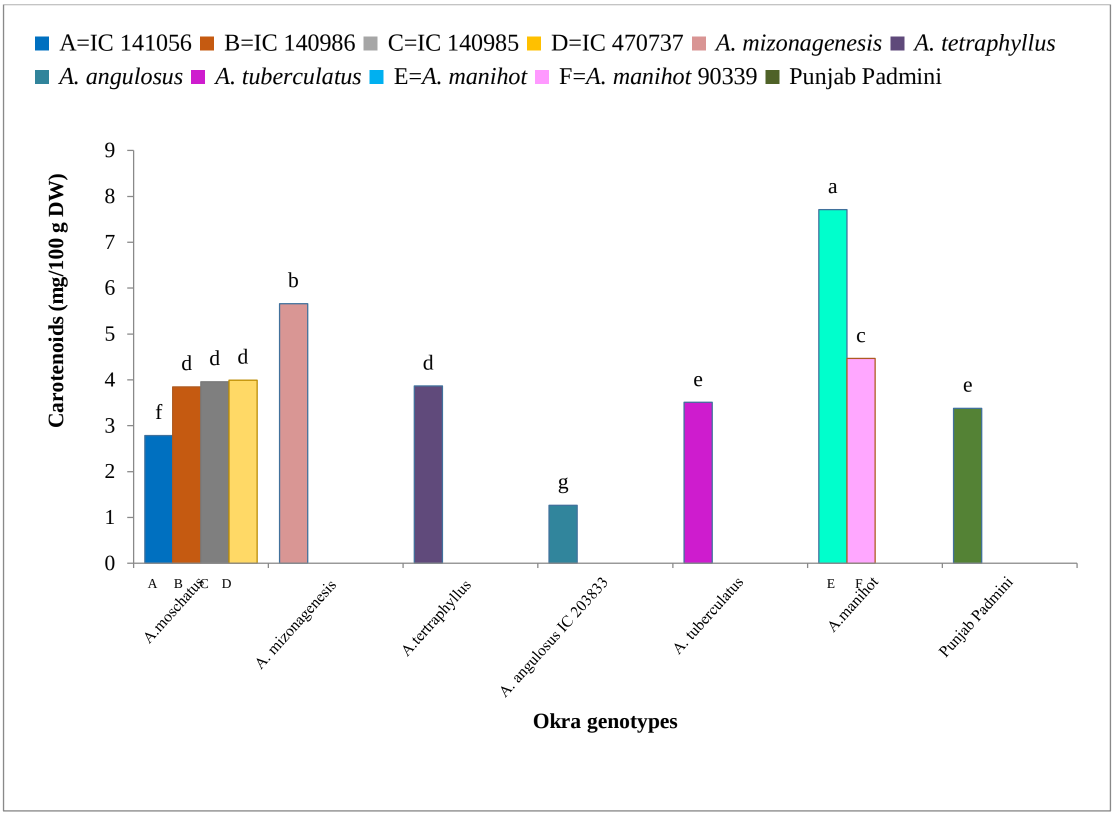

Fig. 4.9(a) Variation in carotenoid content in mature seeds of wild okra

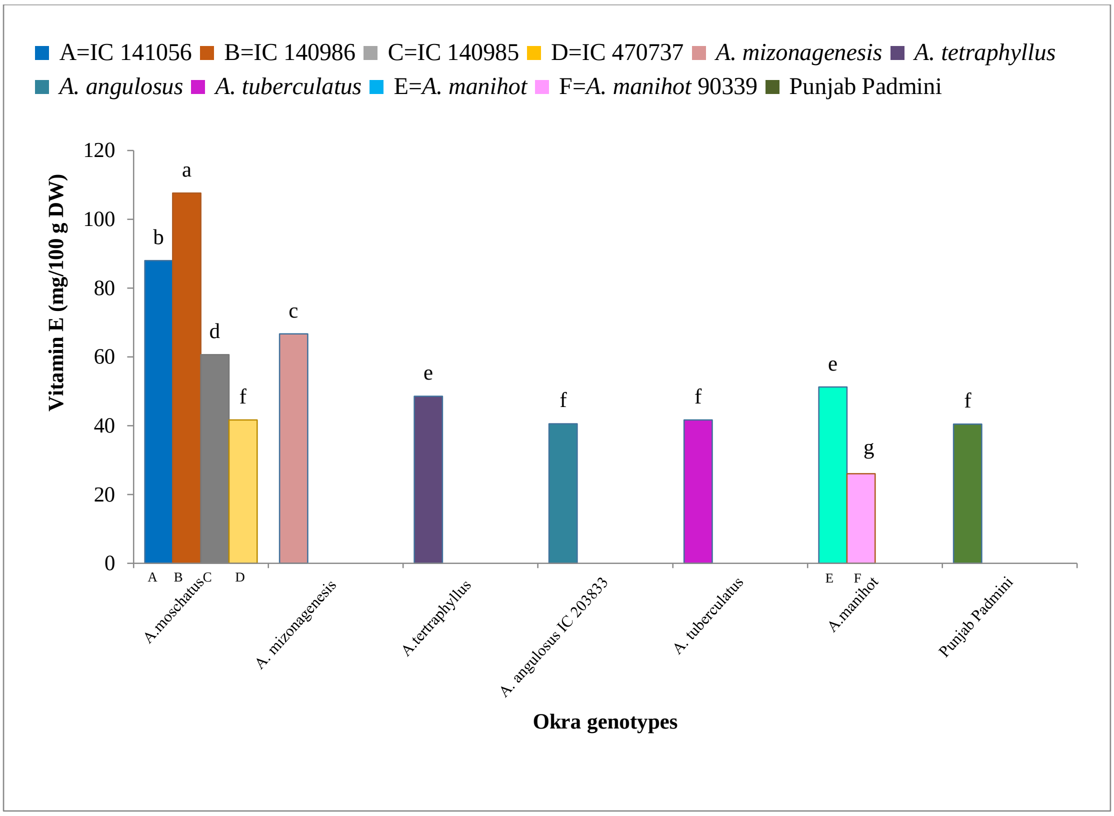

Fig. 4.9(b) Variation in Vitamin E content in mature seeds of wild okra

oil of Sabz Pari and Punjab 8 variety of okra ranged 656.0 and 700.80mg/kg respectively.

Petropoulos *et al* (2017) also reported 26.5 mg/100g DW of total tocopherol content in seeds of "Pylaea" genotype of Greece okra. Kanani *et al* (2019) registerted the vitamin E content in okra seed oil of three Iraqi okra varieties (klpetra, batera and husayniyah) reached 2462.497, 3861.347, 1022.123 ppm respectively.

4.2.4 Minerals

Okra is a good source of minerals. Minerals are necessary for the body and are responsible for structural functions involving the skeleton and soft tissues and for regulatory functions including neuromuscular transmission, blood clotting, oxygen transport, and enzymatic activity. They are classified as macro (>100 mg/day) and micro or trace elements (<100 mg/day). The macro minerals include sodium, potassium, calcium, and phosphorus whereas the trace minerals are: iron, zinc, iodine, copper and manganese.

The data in table 4.10 indicated level of potassium, phosphorus, calcium, iodine, zinc, iron, copper and manganese which varied significantly among different wild species. Potassium act as essential nutrient and helps to regulates the water balance and the acid-base balance in the blood and tissues. It also participates in cellular biochemical reactions, energy metabolism, in synthesis of protein and in normal growth (Hass *et al* 2011). Potassium content in seeds varied from 1173.00- 2772.50 ppm on dry weight basis with the mean value of 2238.76 ppm. Potassium content in cultivated Punjab Padmini was 2780.94 ppm. Among the wild species, the maximum content of potassium was observed in *A. manihot* (2772.50 ppm) followed by its accession IC 90339 (2577.33 ppm) and minimum in *A.moschatus* accession IC 140986 (1173 ppm). Almost all the wild genotypes had value > 2000 ppm except three genotypes *A.moschatus* accession IC 140986, 140985 and *A. mizonagenesis* with value of 1173 ppm, 1938.67 ppm and 1959.52 ppm respectively. However, high concentration of potassium in the body was reported to increase iron utilization (Adeyeye 2002) and was beneficial to people taking diuretics to control hypertension and excessive excretion of potassium through the body fluid (Arinanthan *et al* 2003). Phosphorus, along with calcium, is essential for calcification of bones. Almost 85% of body phosphorus is located in the skeleton and the rest of body phosphorus is needed in soft tissues as a cofactor in enzyme systems which is essential in the metabolism of carbohydrates, lipids, and proteins. In the form of high-energy phosphate compounds, phosphorus contributes to the metabolic potential and also plays an important role in acid/base balance (Kraft 2015). In the present study (Table 4.10) the content of phosphorous ranged from 2584- 7360.3 ppm on dry weight basis. Highest content of phosphorus was found in *A.moschatus* accession IC 140986 (7360.3 ppm). All the wild genotypes had higher phosphorous content than the cultivated genotype. Among the macrominerals, calcium is the most abundant mineral in the human body. It also play vital role in muscle contraction, building strong bones and teeth, blood clotting, regulating heart

Table 4.10 Variation in mineral content in mature seeds of wild okra

Genotypes		Potassium (ppm)	Phosphorus (ppm)	Calcium (ppm)	Iodine (ppm)	Zinc (ppm)	Iron (ppm)	Copper (ppm)	Manganese (ppm)
		Biochemical Parameters							
Abelmoschus moschatus	IC 141056	2316.11±12.36^{f}	6763.3±16.8^{f}	1069.97±6.19^{h}	34.09±2.59^{c}	59.25±0.16^{e}	45.97±0.80^{j}	6.36±0.06^{g}	11.15 ± 0.04^{f}
	IC 140986	1173±4.58^{i}	7360.3±16.6^{a}	1029.13±5.80^{j}	43.10±0.36^{b}	64.66±0.07^{a}	105.36±0.06^{a}	11.37±0.08^{a}	15.34 ± 0.04^{b}
	IC 140985	1938.67±13.80^{h}	6987±5.0^{d}	1082.33±4.51^{h}	36.19±3.16^{c}	62.55±0.05^{d}	56.56±0.06^{d}	7.85±0.05^{d}	12.44 ± 0.04^{e}
	IC 470737	2168.33±8.50^{g}	7150.3±6.7^{b}	1049.33±5.13^{i}	30.17±0.23^{d}	63.74±0.04^{b}	59.75±0.05^{c}	8.28±0.08^{c}	13.23 ± 0.03^{d}
Abelmoschus mizonagenesis		1959.52 ± 6.37^{h}	7085.3±7.5^{c}	1668.63±9.24^{b}	50.42±0.84^{a}	63.45±0.04^{c}	81.71±0.10^{b}	11.36±0.07^{a}	13.44±0.04^{c}
Abelmoschus tetraphyllus		2628 ± 13^{b}	6591.7±3.1^{g}	1441.33±6.03^{c}	24.53±1.22^{e}	52.37±0.03^{f}	49.72±0.06^{g}	8.84±0.05^{b}	8.24±0.04^{g}
***Abelmoschus angulosus var.grandiflorus* IC 203833**		2472.89 ± 2.17^{d}	6766±5.6^{f}	1330.67±5.86^{d}	20.85±0.08^{e}	59.40±0.07^{e}	55.64±0.04^{e}	7.86±0.06^{d}	7.66±0.06^{h}
Abelmoschus tuberculatus		2363 ± 6.24^{e}	6855.7±11^{e}	1286±1^{e}	45.13±0.30^{b}	48.72±0.06^{h}	47.93±0.04^{i}	6.93±0.03^{f}	6.94±0.04^{i}
	–	2772.50±5.77^{a}	2678.6±10^{h}	1112.47±5.28^{g}	24.49±0.05^{e}	28.12±0.10^{j}	48.57±0.06^{h}	4.85±0.04^{i}	5.55 ± 0.05^{k}
Abelmoschus manihot	*Abelmoschus manihot sp. IC 90339*	2577.33±14.01^{c}	2584±3.6^{i}	1148.67±3.51^{f}	24.41±0.06^{e}	29.18±0.04^{i}	52.03±0.05^{f}	5.35±0.05^{h}	6.25 ± 0.04^{j}
Average		2238.76	6082.22	1221.85	33.34	53.14	60.32	7.90	10.02
Abelmoschus esculentus	Punjab Padmini	2780.94±12.42^{a}	546.6±6.2^{j}	2774.63±4.05^{a}	42.08±0.56^{b}	51.34±0.04^{g}	51.37±0.07^{f}	7.63±0.04^{e}	22.94±0.04^{a}
CD (5%)		0.17	0.13	0.13	0.19	0.79	0.41	0.16	0.11

Each value is a mean ± SD of three replications

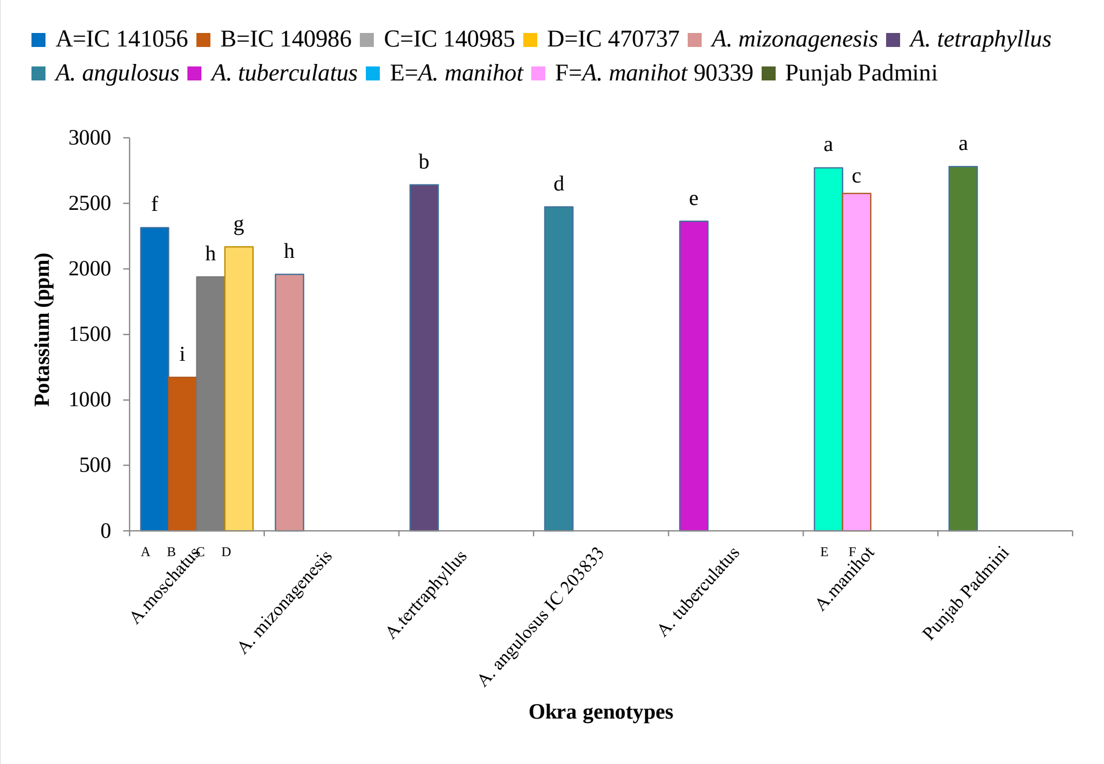

Fig. 4.10(a) Variation in potaasium content in mature seeds of wild okra

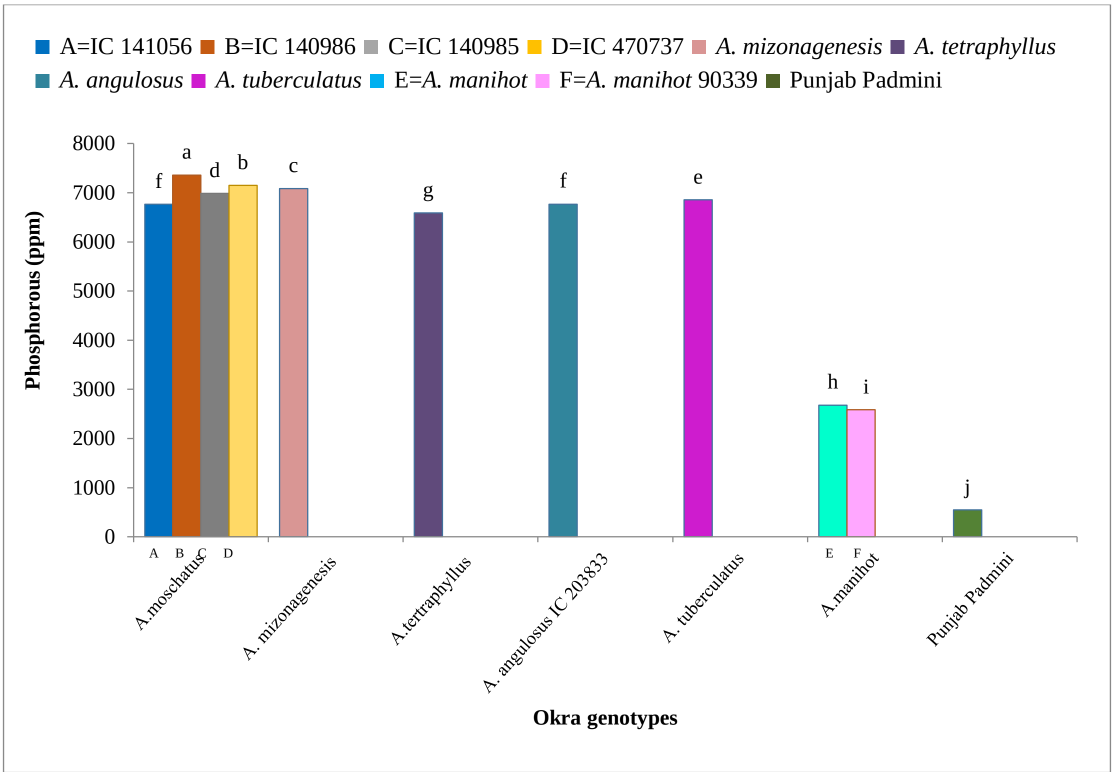

Fig. 4.10(b) Variation in phosphorous content in mature seeds of wild okra

beat and in signalling pathways (Pravina *et al* 2013).The content of calcium in mature wild seeds varied from 1029.13- 1668.63 ppm on dry weight basis with mean value of 1221.85 ppm while the cultivated okra (Punjab Padmini) had content of 2774.63 ppm (Table 4.10). Maximum value of calcium was found in *A.mizonagenesis* followed by *A. tetraphyllus, A.angulosus* 203833 and *A. tuberculatus* with value of 1668.63 ppm, 1441.33ppm, 1330.67 ppm and 1286 ppm respectively. Dietary deficiency or abnormal losses of calcium from the body, can cause severe demineralization of bone or hypocalcemia.

Okra is good source of iodine which prevents goitre and is often recommended by nutritionists (Singla *et al* 2018). In mature seeds of wild okra genotypes iodine content ranged from 20.85- 50.42 ppm on dry weight basis with average value of 33.34 ppm. Punjab Padmini had content of 42.08 ppm. Highest contet of iodine was found in *A. mizonagenesis* and *A.tuberculatus* with value of 50.42 ppm and 45.13ppm respectively. Four genotypes; *A. tetraphyllus*, *A. angulosus* 203833, *A. manihot and A. manihot* IC 90339 were at par for iodine (24 ppm). Zinc is found in cells throughout the body. It play role in the body's defensive (immune) system, cell division, cell growth, wound healing and also in the breakdown of carbohydrates. Table (4.10) also depicted the zinc content in okra seeds. It varied from 28.12- 64.66 ppm on DW basis with mean value of 53.14 ppm in wild okra mature seeds. Maximum content was observed in *A. moschatus* IC 140986 (64.66 ppm) and minimum in *A. manihot* (28.12 ppm). Almost all the wild genotypes had value greater than 50 ppm except *A. manihot* and *A. tuberculatus.* The cultivated genotype Punjab Padmini had zinc content (51.34 ppm). Iron mainly exists in complex forms bound to protein (hemoprotein) as heme compounds (hemoglobin or myoglobin), heme enzymes, or nonheme compounds (flavin -iron enzymes, transferring, and ferritin). It required for the synthesis of oxygen transport proteins (hemoglobin and myoglobin), for the formation of heme enzymes and other iron-containing enzymes involved in electron transfer and oxidation-reductions (Abbaspour *et al* 2014).The iron content in wild mature seeds of okra varied from 45.97- 105.36 ppm on dry weight basis with mean value of 60.32 ppm. Maximum iron content was found in *A. moschatus* accession IC 140986 i.e 105.36 ppm. Copper is an essential trace mineral that work with iron to form red blood cells It helps to keep the blood vessels, nerves, immune system healthy and also aids in iron absorption.The table 4.10 depicted that copper content in wild okra ranged from 4.85- 11.37 ppm on dry weight basis with mean value of 7.90 ppm while cultivated Punjab Padmini had content of 7.63 ppm. Two genotypes namely *A. moschatus* accession IC 140986 and *A. mizonagenesis* contain maximum copper content. Manganese is a trace element that act as cofactor for many enzymes, involved in amino acid and carbohydrate metabolism, bone formation and scavenge reactive oxygen species (Li *et al* 2018) It also plays a role in blood clotting and hemostasis in conjunction with vitamin K (Aschner *et al* 2005). The content of manganese in wild okra genotypes varied from 5.55-

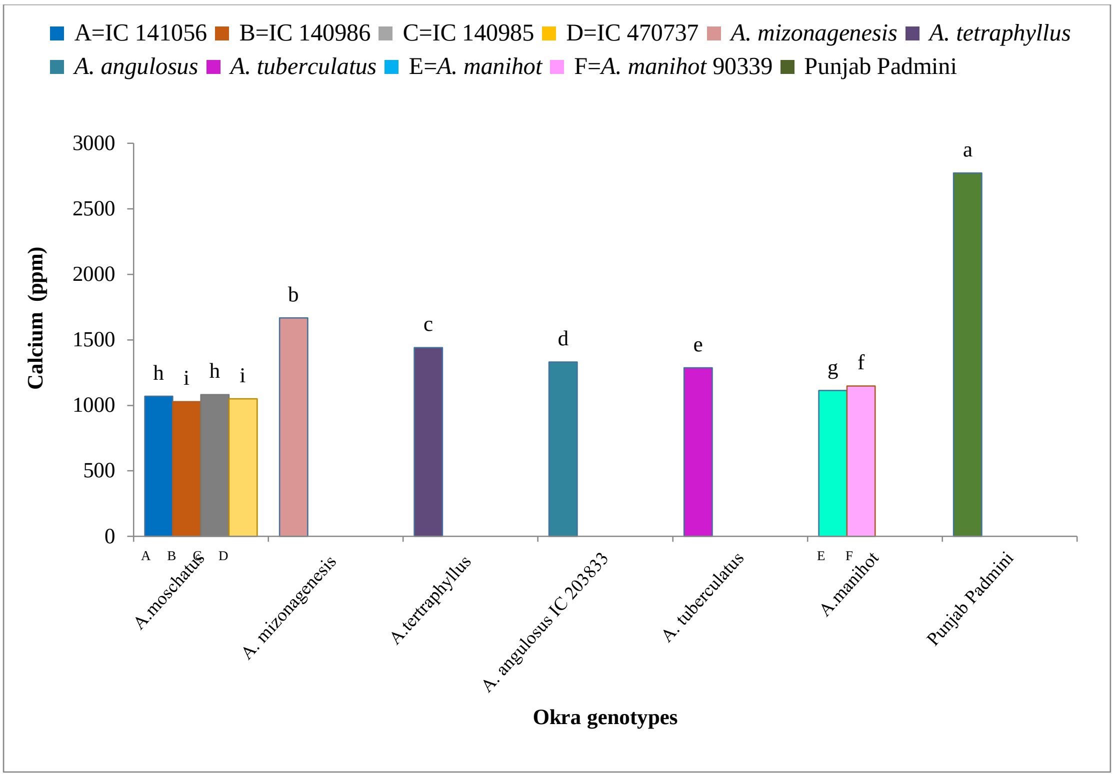

Fig. 4.10(c) **Variation in calcium content in mature seeds of wild okra**

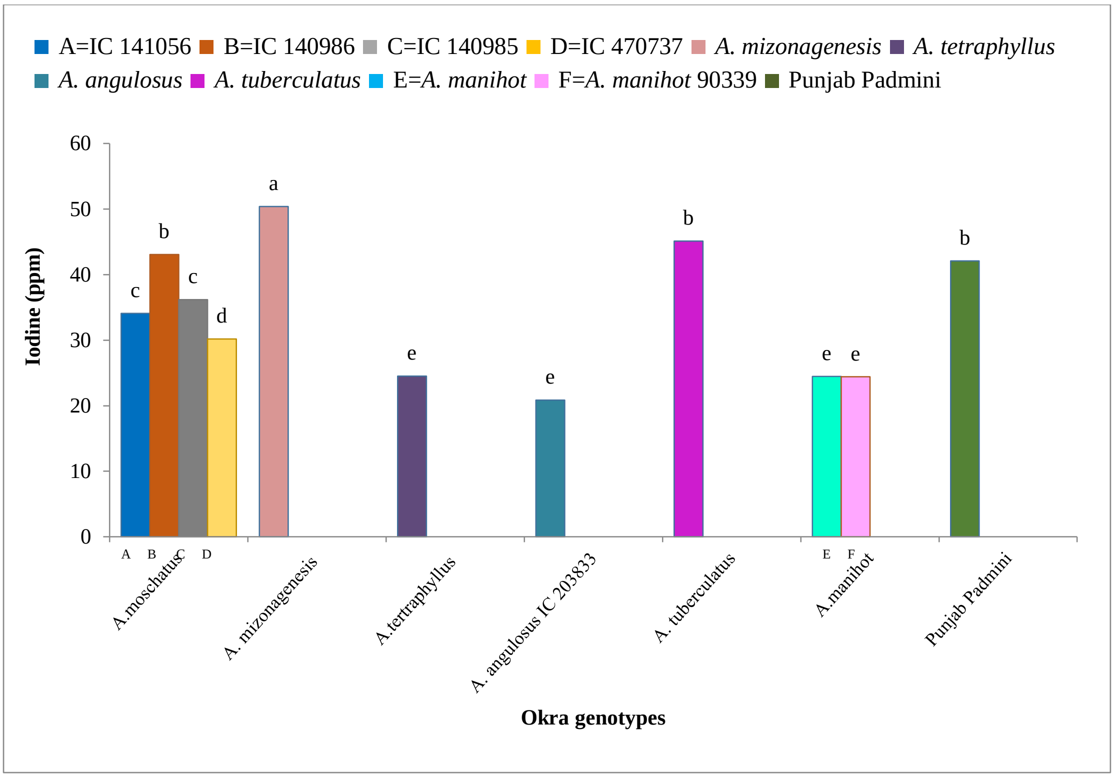

Fig. 4.10(d) **Variation in iodine content in mature seeds of wild okra**

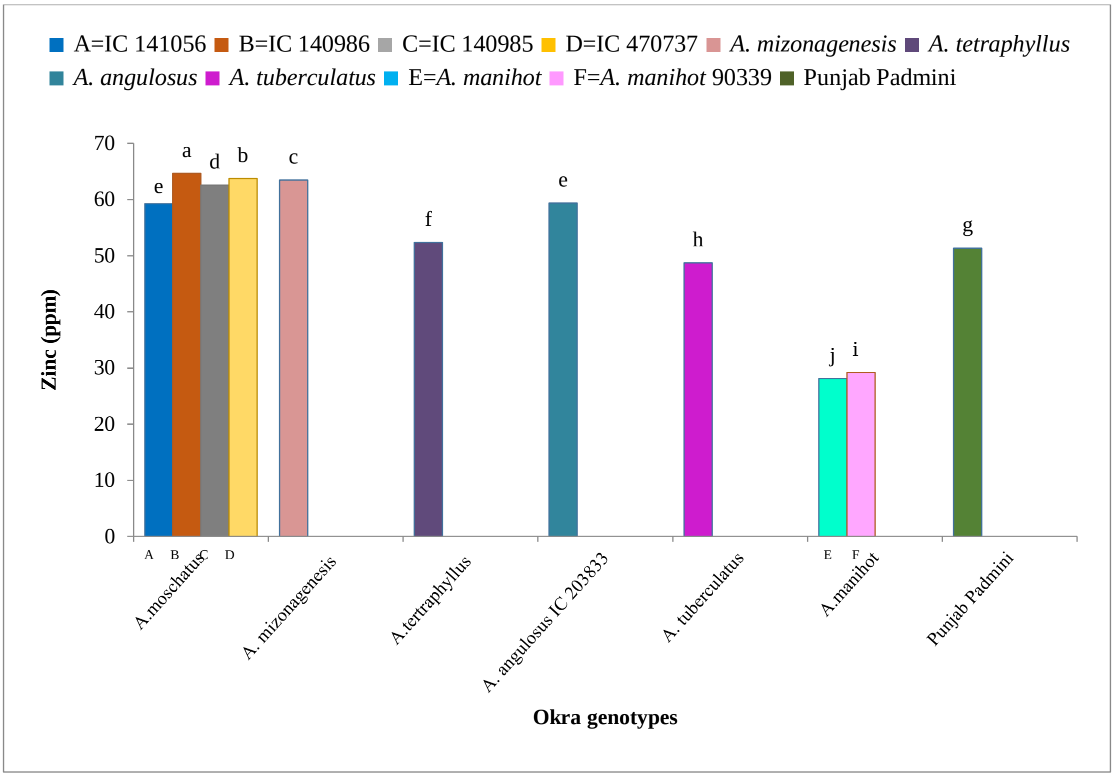

Fig. 4.10(e) **Variation in zinc content in mature seeds of wild okra**

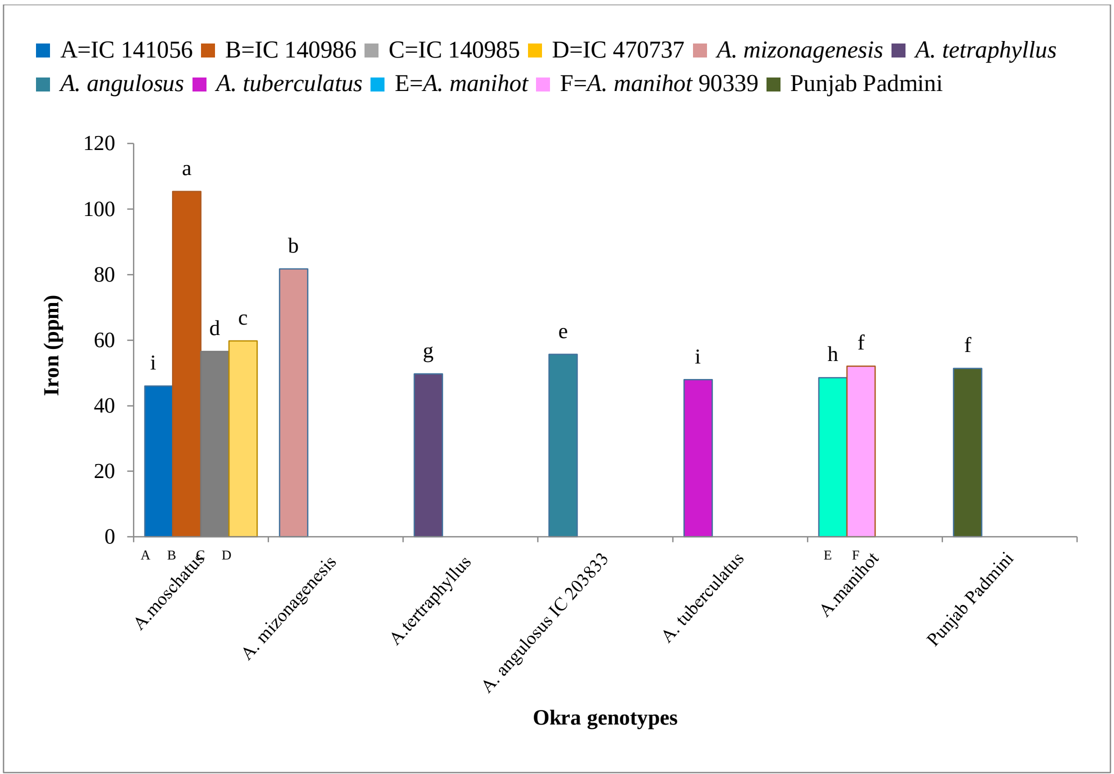

Fig. 4.10(f) **Variation in iron content in mature seeds of wild okra**

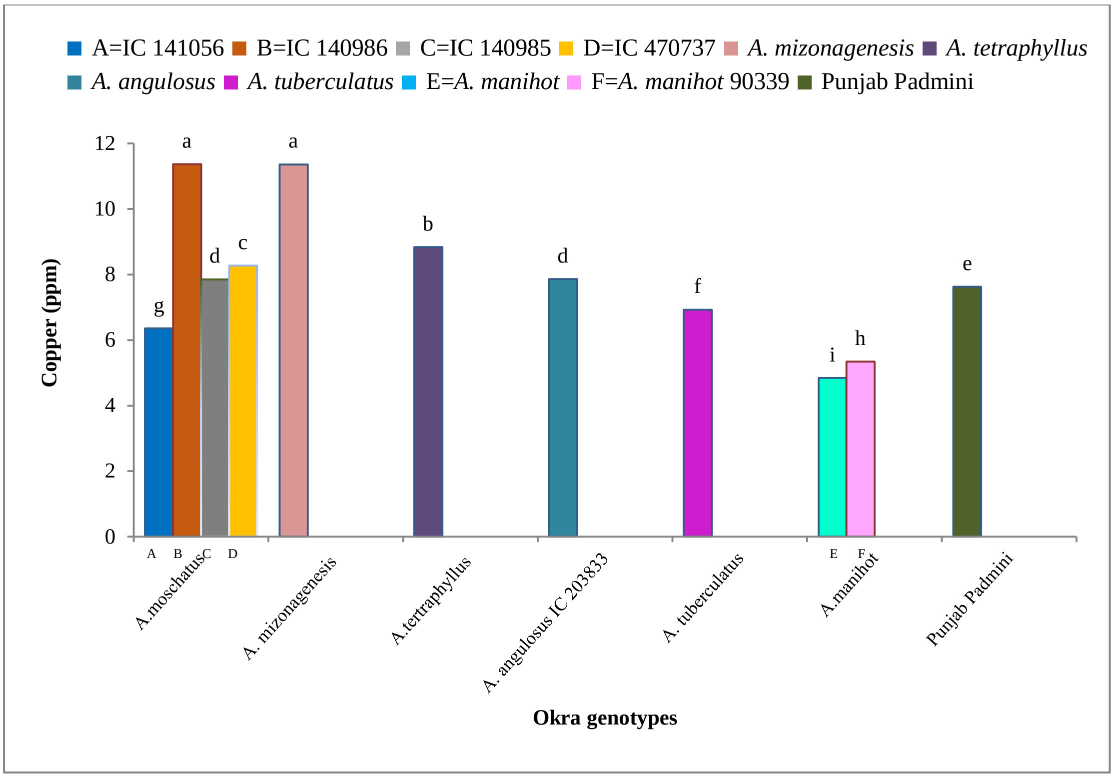

Fig. 4.10(g) Variation in copper content in mature seeds of wild okra

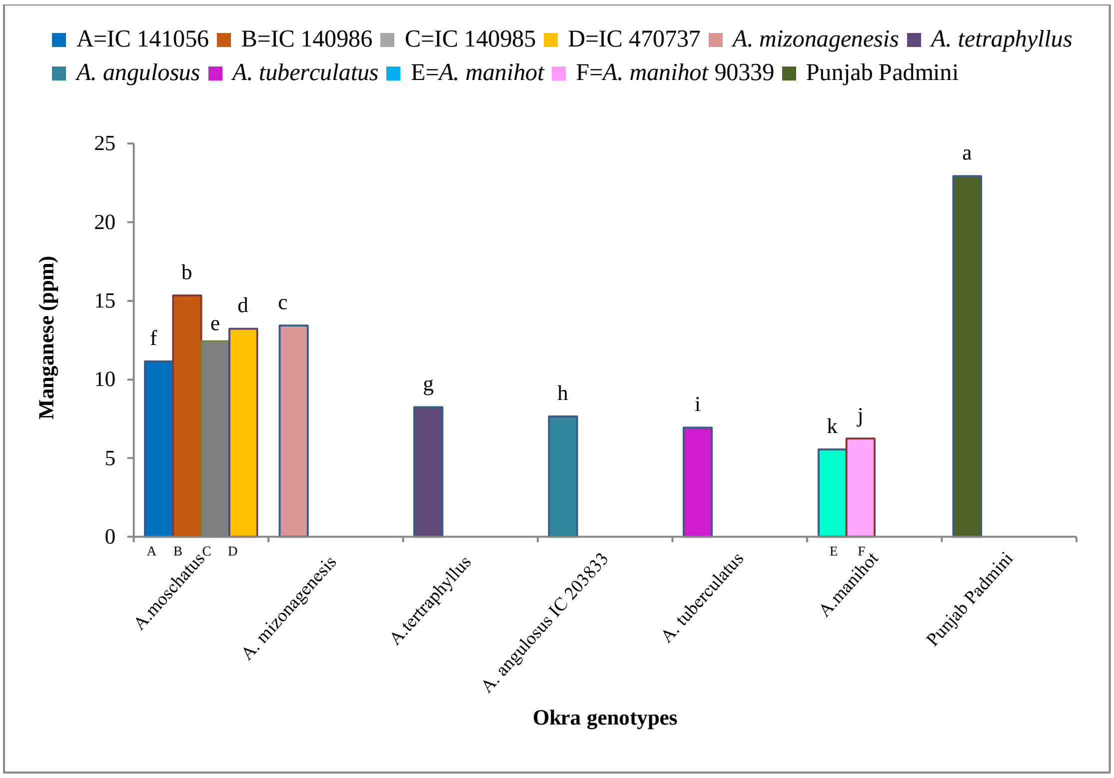

Fig. 4.10(h) Variation in manganese content in mature seeds of wild okra

15.34 ppm on dry weight basis with mean value of 10.02 ppm. Among all the wild species, the accessions of *A. moschatus* had highest content of manganese while lowest was found in *A. manihot*. It was also observed that the cultivated genotypes Punjab Padmini (22.94 ppm) had greater content of manganese than the the wild genotypes. Kanani *et al* (2019) reported the content of minerals (K, Mg, Ca, Fe, Zn, Na and P) in three varieties (*Petra, batera, husayniyah*) of okra seed oil. The result depicted that batera seed oil had maximum concentration of K and Mg with value of 171.33 and 362.76 ppm, respectively while highest calcium concentration was observed in Husayniyah seed oil followed by Petra and batera with value of 12.56 ppm, 7.126 ppm and 2.29 ppm respectively. Iron content was significantally different in three varieties and highest in Petra (47.76 ppm) while zinc concentration was highest in husayniyah i.e 2.5 ppm. The sodium did not show any significant difference between three varieties of okra seed oil but with respect to the phosphorus content, batera seed oil had highest content 393.47 ppm. Ndangui *et al* 2010 studied that content of okra seeds of P 1450, Mg 3259.64, K 109.76, Ca 78.65, Na 54.78 mg /100 g. Pascal *et al* 2018 reported the zinc amd iron content in *A.caillie* (V_{55} V_{57}, V_{58} and V_{60}) and *A. moschatus* (V_{61}). Varieties V_{58} (58 mg/kg) and V_{55} (36 mg/kg) showed the maximum contents of zinc and iron respectively. The copper content also varied from 10 mg/Kg to 12 mg/kg. Petropoulos *et al* 2018 reported the mineral composition in seeds of *Abelmoschus esculentus* genotypes. Results showed that Boyati had the highest content in Ca (3052.0 mg/100 g), Fe (7.51 mg/100 g) and Zn (7.78 mg/100 g) while Lasithi, Veloudo and Choppee also a good source of Mg (600.0 mg/100 g), Mn (1.01 mg/100 g) and K (4000 mg/100 g) respectively.

Correlation Analysis

Correlation analysis is represented in Table (4.11) and was calculated using Excel tool pak. Carbohydrates exhibited significant positive correlation with soluble sugars (r = 0.923), reducing (r = 0.888) and non-reducing sugars (r = 0.893) but negative correlation with mucilage (r = -0.670), dry matter (r = -0.902), chlorophyll and carotenoids. Thus as the amount of dry matter increases, the level of carbohydrates and sugars decreases. Golubkina *et al* (2018) reported significant positive correlation between dry matter and disaccharides (r = 0.98 at $P < 0.01$), but negative correlation (r = −0.86 at $P < 0.05$) between dry matter and monosaccharides. Total soluble protein exhibited significant positive correlation with crude protein (was r = 0.883**) and free amino acids (r = 0.720*). Yora *et al* (2018) also observed positive correlation between chlorophyll-b and total chlorophyll and carotene. The increase in the level of these photosynthetic pigments will result in increased accumulation of photosynthates for higher yield. Flavanols registered significant positive correlation with chlorophyll, carotenoids, total phenols, ascorbic acid and

Table 4.11 Correlation between nutritional, antioxidant and antinutritional parameters in okra pod species and accessions

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15
P1	1														
P2	0.92**	1.000													
P3	0.888**	0.921**	1.000												
P4	0.893**	0.985**	0.842**	1.000											
P5	-0.670*	NS	NS	NS	1.000										
P6	NS	NS	NS	NS	NS	1.000									
P7	-0.902**	-0.857**	-0.788**	-0.845**	0.661*	NS	1.000								
P8	NS	NS	NS	NS	NS	NS	NS	1.000							
P9	NS	NS	NS	NS	NS	NS	NS	NS	1.000						
P10	NS	NS	NS	NS	NS	NS	NS	NS	0.883**	1.000					
P11	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.720*	1.000				
P12	-0.685*	NS	NS	-0.623*	0.793**	NS	0.683*	NS	NS	NS	NS	1.000			
P13	-0.642*	NS	NS	-0.612*	0.799**	NS	0.736**	NS	NS	NS	NS	0.969**	1.000		
P14	-0.685*	-0.603*	NS	-0.627*	0.799**	NS	0.698*	NS	NS	NS	NS	0.999**	0.978**	1.000	
P15	-0.756**	-0.667*	NS	-0.679*	0.762**	NS	0.750**	NS	NS	NS	NS	0.980**	0.936**	0.978**	1.000
P16	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
P17	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
P18	NS	NS	NS	NS	0.644*	NS	NS	NS	0.657*	0.675*	NS	0.877**	0.825**	0.871**	0.819**
P19	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.612*	NS	NS	NS	NS
P20	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.636*	NS	0.760**	0.698*	0.750**	0.698*
P21	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
P22	0.615*	NS	0.730*	NS	NS	NS	NS	-0.656*	NS	-0.659*	NS	NS	NS	NS	NS
P23	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.633*	NS	NS
P24	NS	NS	NS	NS	NS	NS	NS	-0.665*	NS	NS	NS	NS	NS	NS	NS
P25	NS	NS	NS	NS	NS	NS	0.782**	NS	NS	NS	NS	NS	NS	NS	NS
P26	0.815**	0.706*	0.640*	0.700*	-0.658*	NS	-0.800**	NS	NS	NS	NS	-0.648*	-0.619*	-0.655*	-0.747**

	P16	P17	P18	P19	P20	P21	P22	P23	P24	P25	P26
P16	1.000										
P17	NS	1.000									
P18	0.630*	NS	1.000								
P19	NS	NS	NS	1.000							
P20	0.780**	NS	0.726*	NS	1.000						
P21	NS	NS	0.605*	NS	NS	1.000					
P22	NS	0.767**	NS	NS	NS	NS	1.000				
P23	NS	NS	NS	NS	NS	NS	NS	1.000			
P24	NS	0.842**	NS	NS	NS	NS	0.707*	NS	1.000		
P25	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.000	
P26	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.744**	1

NS represent non-significant coefficients, P1, carbohydrates; P2, total soluble sugars; P3, reducing sugars; P4, non-reducing sugars; P5, mucilage;P6, crude fibers; P7, dry matter; P8, ash content; P9, crude protein; P10, total soluble protein; P11, free amino acids; P12, chlorophyll a; P13, chlorophyll b; P14, total chlorophyll; P15, carotenoids; P16, total phenols; P17, 0-dihydroxyphenols; P18, flavanols; P19, vitamin E; P20, vitamin C; P21, DPPH; P22, FRAP; P23, phytate; P24, tannins; P25, oxalate; P26, saponins.

** Correlation is significant at 0.01 level (2-tailed), *Correlation is significant at 0.05 level (2-tailed)

DPPH. Mohite and Gurav (2019) reported that total phenolic content was positively correlated with total flavonoids (r =0.666) and DPPH activity (r = 0.724). Saponins exhibited negative correlation with chlorophyll, carotenoids and oxalate while phytate depicted negative correlation only with chlorophyll b (r = -0.633).

Elite genotypes with favourable combination of nutrients, anti-nutrients and antioxidants are depicted in table.4.12 (a) for the immature pods and 4.12 (b) for the mature seeds. *A. moschatus* IC 470737 was rich in carbohydrate components while IC 140986 was rich in vitamin E (Table.4.12 (a)). *A manihot* harboured high antioxidants, protein components and mucilage while harboured low tannins and saponins. *A. tetraphyllus* exhibited hish antioxidants and low phytate and oxalate. And *A. tuberculatus* depicted high mucilage and low phytate and saponin. For seeds, *A. moschatus* seeds were rich in minerals, vitamin E and oil had high oleic and linoleic acid (Table 4.12 (b)). *A. tuberculatus* seeds had high protein components and *A. mizonagenesis* harboured high oil content.

Table 4.12(a): Elite genotypes harbouring good nutrient composition, antinutrients and antioxidants in pods

Genotypes	Biochemical Parameters
A.moschatus accession IC 470737	Total carbohydrates↑, Total soluble sugars↑, Reducing sugars↑, Non-reducing sugar↑
A.moschatus accession IC 140986	Vitamin E↑
A.manihot L. Medik	Chlorophyll a↑, Total chlorophyll↑, Carotenoids↑, Ascorbic acid↑, Crude protein↑, Total soluble protein↑,Tannins↓, Saponin↓
A.manihot accession IC 90339	Mucilage↑, Ash content↑, Chlorophyll b↑, Flavanols↑, DPPH activity↑, Tannins↓, Saponin↓
A. tetraphyllus	Phenols↑, o-dihydroxyphenols↑, FRAP activity↑, Phytate↓, Oxalate↓
A. tuberculatus	Mucilage↑, Dry matter↑, Phytate↓, Saponin↓

Table 4.12(b): Elite genotypes harbouring good nutrient components in mature seeds

Genotypes	Biochemical parameters
A. moschatus accession IC 140986	Vitamin E, Oleic acid, P, Zn, Fe, Cu, Mn
A. moschatus accession IC 470737	linoleic acid
A. manihot (L.) Medik	Carotenoid content, Potassium
A. tuberculatus	Crude protein, Total soluble protein
A. mizonagenesis	Oil content

CHAPTER V

SUMMARY

Okra (*Abelmoschus esculentus*) is an important vegetable crop grown mainly in tropical and subtropical parts of the world. It is mainly grown for immature pods but all parts are consumed by humans either as food or medicine. Immature pods are consumed as vegetable, in salads, soups and stews. The crop is endowed with various health benefits and has industrial applications too. Okra fruit contain carbohydrates, minerals, vitamins, fibre and antioxidants that play vital and protective role for human body. Seeds contain good quantity of oil and good quality protein. Okra yield and quality are affected by many biotic stresses. Wild relatives are potential source for stress resistance and bioactives for cultivated cultivars. The resilience could be due to higher content of bioactive molecules; antioxidants and polyphenols. The wild accessions also exhibit efficiency for inputs and productivity. The high nutrient value of many wild plants make them important from human nutrition point of view. *Abelmoschus* spp. include many wild species viz, *A. manihot* (L.) Medik, *A. moschatus* (L.) Medik, *A. tuberculatus*, *A. manihot* (L.) Medik subsp. *tetraphyllus*, *A. mizonagenesis*, *A. angulosus* etc. The nutraceutical properties of the wild *Abelmoschus* species may benefit human health. In the present study the immature fruits and seeds of okra genotypes (wild and cultivated) were evaluated for various biochemical traits. The immature pods were taken after 45 days of sowing from fields of Department of Vegetable Science, Punjab Agricultural University, Ludhiana and the seeds were taken from mature pods. The pods were analysed for moisture, carbohydrates, total soluble sugars, reducing sugars, non-reducing sugars, mucilage, crude protein, phenolics, carotenoids, chlorophyll, tocopherols, vitamin C, total antioxidant activity and antinutrients (oxalate, phytate, saponins, tannins). The seeds were analysed for total soluble proteins, total free amino acids, oil and fatty acid composition, ash and minerals. Total carbohydrate content in immature pods varied from 22.34- 93.10% on DW basis in wild okra with maximum content in *A. moschatus spp. Moschatus Medik* IC 470737 and *A. tetraphyllus*. The cultivated genotype Punjab Padmini harboured 88.25% carbohydrate content. Total soluble sugars (TSS) ranged from 13.74- 84.36% while reducing sugars registered variation of 0.17- 19.41% on DW basis with the mean value of 6.79% while the non-reducing sugar content ranged from 31.25- 64.95%. *A. moschatus* (IC 470737) registered 84.36% TSS while minimum content was observed in *A. tuberculatus.* Total soluble sugar content of Punjab Padmini was 68.96% DW and it had higher content of reducing sugars (10.69%) and non-reducing sugars (58.27%) in comparison to average of wild genotypes. *A. moschatus* accession IC 470737 exhibited maximum content of reducing as well as non-reducing sugars. Minimum non-reducing sugar content was found in *A. tuberculatus*

(13.47%). *A. moschatus* accession IC 470737 represented maximum amount of total carbohydrates, total soluble sugars as well as reducing sugar and non-reducing sugars. The wild okra pods depicted mucilage in the range of 6.64 (*A. moschatus* accessions IC 140985)- 19.64% (*A. manihot* (90339)) on DW basis while Punjab Padmini had content of 6.56%. The wild genotypes; *A. moschatus* (IC 141056 and IC 470737), *A. mizonagenesis*, *A. angulosus* (IC 203833), *A. tuberculatus* and *A. manihot* (L.) Medik possessed mucilage content of 10.27%, 16.07%, 13.84%, 13.24%, 18.81% and 17.11% respectively. The crude fiber varied from 11.41- 29.53% on DW basis with mean value of 20.25% in wild okra pods. Maximum content (29.53%) was found in *A. angulosus var. grandiflorus* IC 203833. Most of the wild genotypes had higher content than Punjab Padmini. Dry matter content ranged from 11.18- 27.59% on FW basis. Punjab Padmini contained dry matter content of 10.41%. *A. moschatus* IC 470737 had minimum dry matter content (11.18%) while highest was observed in *A. tuberculatus* (27.59%). Ash content in wild okra pods ranged from 1.10- 6.94% on FW basis. Maximum ash content was registered in *A.manihot* accession IC 90339. Cultivated okra; Punjab Padmini harboured 5.79%. The crude protein in immature fruits of wild okra varied from 2.13- 6.32% on FW basis. The cultivated genotype had greater content of crude protein than the average of wild genotypes. Total soluble protein range detected was 0.93- 3.56% on FW basis. The average of wild genotypes was less than the content observed in Punjab Padmini (0.27%). Free amino acid content in wild okra pods depicted a range from 0.16- 0.27% on fresh weight basis. Punjab Padmini depicted value higher than wild okra. Maximum content was observed in *Abelmoschus manihot* (L.) Medik. The content of chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and vitamin C varied significantly among wild okra species. The chlorophyll a content in pods of wild okra varied from 13.07- 38.63 mg/100 g FW. Chlorophyll b content ranged from 1.68- 9.04 mg/100 g FW and total chlorophyll content from 15.84-52.83 mg/100 g FW. *Abelmoschus manihot* (L.) Medik and *Abelmoschus manihot sp.* accession IC 90339 had a maximum content of chlorophyll a, chlorophyll b and total chlorophyll. Punjab Padmini registered lower content of chlorophyll a (6.68 mg/100 g FW), chlorophyll b (0.69 mg/100gFW) and total chlorophyll (8.35 mg/100 g FW) than average of wild okra. Total carotenoid content in wild okra pods ranged from 4.48- 13.25 mg/100 g FW while Punjab Padmini recorded 1.86 mg/100 g FW. Highest content was observed in *A. manihot* (L.) Medik (13.25 mg/100 g FW). The phenolic content varied from 122.95- 412.01 mg/100 g DW. *A. tetraphyllus* registered highest phenolic content followed by *A.manihot* accession 90339, *A. manihot* (L.) Medik, *A. mizonagenesis*. Punjab Padmini exhibited content of 213.61 mg/100 g DW. The content of O-dihydroxyphenols and flavonols in wild okra pods ranged from 4.30- 17.99 mg/100g, 63.63- 274.49 mg/100g DW respectively. The cultivated okra exhibited 6.08 mg/100g of O-dihydroxyphenol and 134.93 mg/100g DW of Flavonol content. Highest content was recorded in *A. tetraphyllus* (17.99 mg/100g) and *A. manihot* sp. IC 90339 (274.49 mg/100g) respectively. Vitamin E content varied from 15.14- 51.24 mg/100 g DW. Punjab Padmini recorded 23.12mg/100 g Vit E content. Maximum content was observed in *A. moschatus* accession IC 140986. Vitamin C in wild okra ranged from 41.26- 120.10 mg/100 g FW. Minimum amount was observed in *A.moschatus* IC 470737 and maximum in *A. manihot* (L.) Medik while Punjab Padmini registered 61.30 mg/100 g content. DPPH radical scavanging activity depicted variation of 39.74- 62.90% whereas FRAP activity ranged from 4.54- 11.22 mg AAE/g DW. *A. manihot* (IC 90339) registered maximum DPPH activity. Maximum FRAP value was observed in *A. tetraphyllus* followed by *A. moschatus* (IC470737). Punjab Padmini recorded DPPH and FRAP activity of 47.97% and 3.78 mg AAE/g. The phytate content ranged from 0.65- 2.08% DW. *A. tetraphyllus* and *A. tuberculatus* registered lowest phytate. Tannins varied from 1.26- 3.30% DW. The oxalate content varied in the range of 0.23- 0.63% DW. *A. tetraphyllus* and *A. mizonagenesis* registered low oxalate content and it was also low in Punjab Padmini. Saponin content in immature pods of wild okra varied from 0.19- 1.04% DW. Punjab Padmini registered content of 1.44%. The crude protein content in wild okra seeds varied from 19.35- 27.09% DW. The total soluble protein ranged from 9.83- 19%. *A .tuberculatus* had the maximum content of both the entities with values of 27.09% and 19% respectively. The cultivated genotype depicted crude protein value of 21.71% and total soluble protein value of 13.16% in its seeds. Total oil content in wild okra seeds ranged from 10.68-27.61% DW with a mean value of 15.56%. Punjab Padmini registered 8.83% of oil. *A. mizonagenesis* harboured maximum oil content of 27.61%. Seeds registered carotenoids in the range of 1.27- 7.11 mg/100 g DW. Punjab Padmini depicted 3.38% of carotenoids. Maximum amount of carotenoid content was found in *Abelmoschus manihot* (L.) Medik (7.11 mg/100g). Vitamin E content in seeds ranged from 26.08- 107.63 mg/100g DW with average value of 57.29 mg/100g. Minerals were estimated in mature seeds. Among them potassium content in seeds varied from 1173.00- 2772.50 ppm DW. Punjab Padmini exhibited 2780.94 ppm. Maximum content was observed in *A. manihot*. Almost all the wild pods had value > 2000 ppm. Highest phosphorus content was found in *A. moschatus* IC 140986 (7360.3 ppm). Calcium in mature seeds varied from 1029.13- 1668.63 ppm DW basis. Punjab Padmini depicted 2774.63 ppm. Maximum content was found in *A.mizonagenesis*. Highest iodine content was found in *A. mizonagenesis* and *A.tuberculatus* with value of 50.42 ppm and 45.13ppm respectively. Zinc varied from 28.12- 64.66 ppm on DW basis. Maximum was observed in *A. moschatus* IC 140986. Maximum iron content was found in *A. moschatus* IC 140986 i.e 105.36 ppm.

Copper content ranged from 4.85- 11.37 ppm with mean value of 7.90 ppm while Punjab Padmini had 7.63 ppm. *A. moschatus* accession IC 140986 and *A. mizonagenesis* contained maximum copper. Manganese was observed to be maximum in Punjab Padmini (22.94 ppm). In conclusion *A. manihot* and *A. tetraphyllus* pods depicted high antioxidants, mucilage content but harboured low content of tannins and saponins. *A. moschatus* pods were rich in nutrients and its seeds depicted high Vit. E, oleic, linoleic acid and minerals. *A. tetraphyllus* pods possessed high antioxidants and low phytate and oxalate and *A. mizonagenesis* exhibited high oil content in seeds. These wild genotypes can be used in okra improvement programmes.

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