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ORIGINAL ARTICLE **OPEN ACCESS**

Protein profiles at Developmental stages of Oleaginous tea (*Camellia* spp) seed

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ABSTRACT
Tea seeds are reported to be oleaginous. Considering importance of tree borne oil seeds for countries like India, which is not yet self sufficient on edible oil production, the present study was conducted to know the protein profile oleaginous of tea seed during its various stages of development and also to explore probable relationship of proteins with accumulation of oil. SDS-PAGE was used to detect the molecular weight of the extracted proteins at four different developmental stages of the tea seeds of eight bi-clonal tea germplasm. It was observed that the soluble protein content increased gradually from first month to eight months, which is the final stage of fruit development. At all the stages of development, the highest soluble protein percentage was observed in bi-clonal seed stock TS-491, which were 6.41, 10.23, 13.87 and 14.48 % at first, third, fifth and eight months after fruit set, respectively. A common protein band of molecular weight 66 kDa was observed for all the bi-clonal seed stocks at different developmental stages. The correlation studies indicated that there was negative significant correlation (-0.67) between the oil content with respect to soluble protein content of different bi-clonal seed stocks.
Key words: *Tea seeds, bi-clonal, oleaginous, protein profile, SDS-PAGE*

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INTRODUCTION

India, the highest producer of beverage tea (*Camellia* spp), is not yet self sufficient on edible oil production. A large portion of oils from secondary sources is lying unexploited, which also includes potentiality of oil production from tree origin. Since, tea has so far been used only as beverage, the leaves or the new vegetative growth is considered to have economic importance. However, tea seed oil is extracted on commercial scale in some other countries. The kernels, which make up about 70% of the tea seed weight, are rich in oil [4]. Like other genera of *Camellia,* from *Theaceae* family, the tea plant (*C. sinensis*) produces large oily seeds [20, 7, 21].

Proteomic analysis has been reported to become a powerful tool to profile the biological processes of plants [2]. This approach has been widely applied to study the protein changes during seed development for a variety of plants including barley (*Hordeum vulgare*), barrel medic (*Medicago truncatula)*, soybean (*Glycine max*), wheat (*Triticum aestivum*), rapeseed (*Brassica napus*), birdsfoot trefoil (*Lotus japonicas*) and castor (*Ricinus communis*) [23, 8, 9, 11,12, 13, 5].

A few reports related to proteins in tea seed are available. The protein of defatted tea seed meal was reported to be 16.7% [24, 26]. The crude protein of tea seed meal was reported to be 10.5±0.07% [22, 6]. Some storage proteins were reported in *Camellia oleifera* seed such as *oleosin, lipidbodybrane, caleosin, steroleosin, globulin, glutelin* and *albumin* [14, 15]. Alkaline extraction followed by acid precipitation for extraction of protein from tea seed meal with protein recovery of 57.8%was reported [6]. Protein yield was 47.1% at 40 ºC with extraction medium of pH 7 [22]. Differentially expressed proteins in *Camellia sinensis* seed embryos were reported earlier [2]. Earlier, we have reported [21] the quality of tea seed oil of eight tea seed stocks which are commercially utilized for propagation purpose in Assam, India. However, study on protein content in relation to oil accumulation at various stages of tea seed development is yet to be done. Considering importance of exploitation of tree borne oil seeds for countries like India, which is not yet self sufficient in edible oil production, the present study was

conducted to know the protein profiles of tea oleaginous seed during its various stages of development and also to explore probable relationship of proteins with accumulation of oil.

MATERIAL AND METHODS

The fruit of the different clones were collected from the approved seed *bari* of the Tocklai Tea Research Institute, Jorhat, Assam. The clones collected for the present study were TS-462, TS-378, TS-379, TS-491, TS-463, TS-464, TS-506 and TS-520. The first stage was after the first month of fruit development (April), the second stage was after the third month of fruit development, the third stage was after fifth month of fruit development and finally, the fourth stage was eight months after fruit development. The fruit were stored at - 20ºC until these were used for further analysis. The cotyledons, manually separated from the seeds were used for various biochemical analysis. All chemicals were collected from Sisco Research Laboratories Private limited, Andheri, Mumbai, Maharastra, India and these were of analytical grade.

Crude oil content

The crude oil content of the dried cotyledons was determined according to AOAC [1].

Extraction and estimation of soluble protein

Seed protein was extracted according to protocol described by Natarajan *et al.* [18]. The defatted tea seed powder (100mg) was homogenized with 5ml of a solution containing 10% (w/v) TCA in acetone with 0.07% (v/v) 2-mercaptoethanol. The total protein was precipitated for overnight at -20 °C. The extract was centrifuged at 20,800*g* for 20 min at 4 °C. The pellet was washed three times with acetone containing 0.07% (v/v) 2-mercaptoethanol. Then the pellet was dried under vacuum for 30mins, and the acetone dried powder was resuspended in 1ml of lysis buffer (9M urea, 1% Dimethyl[3-(propyl]. azaniumyl}propane-1-sulfonate (CHAPS) buffer, 1% Dithiothreitol, DTT), followed by sonication on ice for 30 min. Insoluble material was removed by centrifugation at 20,800*g* for 20 min at 4 °C. The proteins in the extract were quantitatively determined according to Lowry's Method [17].

The method described by Laemmli, [16] was followed for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to detect the molecular weight of the extracted proteins at four different stages of development of tea seeds. A 10 % separating gel was used for the present study. The gel was stained with coomassie brilliant blue solution. A protein marker of range 14-220 kDa was used as a standard marker.

Statistical analysis

The data obtained from various biochemical analysis (done in triplicates) were subjected to statistical analysis using 'completely randomized design' [19]. Correlation analysis was done by according to Pearson correlation test [19].

RESULTS AND DISCUSSION

Soluble protein content (%, dry basis)

The soluble protein content of defatted sample of different bi-clonal seed stocks at four different developmental stages *i.e.* first, third, fifth and eight months after fruit development are presented at Table.1. It was observed that the soluble protein content increased gradually from first month to eight months, which is the final stage of fruit development. The highest soluble protein percentage was observed in bi-clonal seed stock TS-491, which were 6.41, 10.23, 13.87 and 14.48 at first, third, fifth and eight months after fruit development, respectively. The lowest soluble protein (2.51%) at first month after fruit development was found in TS-462. However, in defatted tea seed cake, the crude protein was reported to be 16.7 [24, 26] and 10.5±0.07% [22]. In the present study, there was a gradual increase in oil content in relation to soluble protein content and the both were found to be the highest at the fully matured stage.

Correlation between soluble protein content and oil content

In the first three stages of fruit development, no oil accumulation was observed in all the bi-clonal tea seed stocks analyzed (Table 1). However, at the final stage of fruit development *i.e.* fully matured stage, the oil content was found to be the highest. At this stage, the oil content of tea seeds ranged between 11.02% and 26.84%. Among the bi-clonal tea seed stocks, TS-379 showed the highest oil content of 26.84% after eight months of fruit development. The bi-clonal tea seed stock TS-491 showed the lowest oil content of 11.02% after eight months of fruit development. Oil content of 23% and 27.21%, were also reported earlier in tea seeds [25, 15]. However, higher oil content (30.5%) was reported in tea seeds collected from Iran [20].

The correlation studies indicated that there was negative significant correlation (-0.67) between the oil content with respect to soluble protein content of different bi-clonal seed stocks.

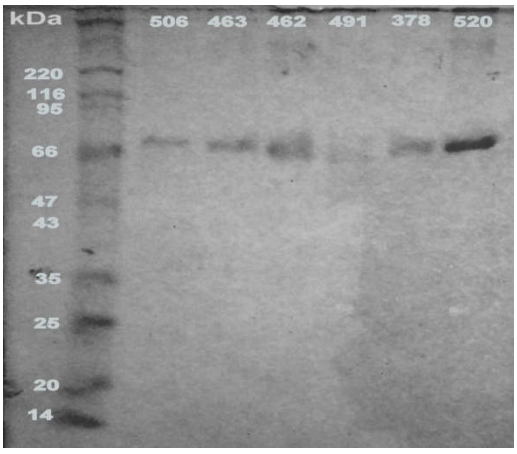
Protein profiles

The soluble protein extract of different bi-clonal tea seed stocks prepared at various developmental stages was subjected to SDS-PAGE (10% resolving gel) to know the molecular weight of proteins, the banding patterns of which are presented at Fig.1. In the present study, differences in protein bands were observed among different biclonal seed stocks. A common protein band of molecular weight 66 kDa was observed for all the bi-clonal seed stocks at all the stages of fruit development. However, at third month after fruit development, together with the protein band of molecular weight 66 kDa, an additional band of molecular weight of 20 kDa, was observed for bi-clonal seed stock TS-463. Protein bands of molecular weight 35 kDa and 47 kDa along with band of molecular weight 66 kDa were observed in TS-463 at eight month of fruit development. However, protein bands of molecular weight 47 kDa along with band of molecular weight of 66 kDa were observed in TS- 491 and TS-506 at eight month of fruit development. The molecular weight of the proteins detected in the present study were found to be approximately similar to some of the proteins {Oleosins (16-24 kDa), Caleosins (24 kDa), Keto acyl CoA reductase (36kDa), Acetyl CoA carboxyl transferase alpha subunit (35.24 kDa), Biotin carboxylase (49.32 kDa) and Glycerol-3-phosphate acyl transferase (66.51 kDa)} to be involved in triacylglycerol (oil) biosynthesis.
Earlier, SDS-PAGE analysis (15% resolving gel) in tea seed revealed proteins of molecular mass ranging from 21 to 43 kDa and after anion exchange chromatography, intense protein bands at 14 kDa, 23 kDa, 28 kDa, 31 kDa and 33 kDa were also observed [22]. The differences in protein banding patterns of present study with that of reported earlier [22] might be due to differences in the concentration of resolving gel.

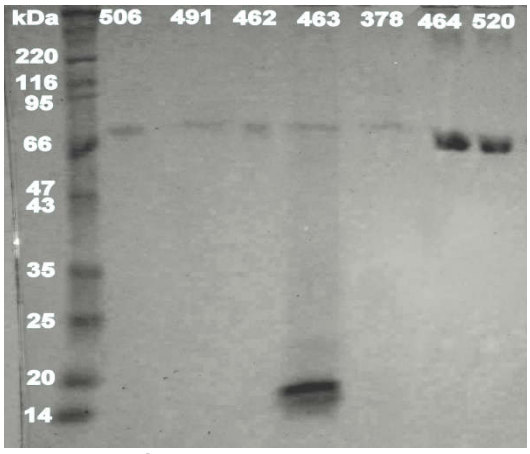
Table 1 - Soluble protein content (% of defatted cotyledons) and oil content in dried cotyledons of different bi-clonal tea seed stocks

Bi-clonal seed stock	A		B		C		D	
	Soluble protein	Oil content	Soluble protein	Oil content	Soluble protein	Oil content	Soluble protein	Oil content
TS-378	4.55	ND	8.73	ND	10.92	ND	11.41	19.96
TS-379	-	ND	-	ND	-	ND	6.49	26.84
TS-462	2.51	ND	4.83	ND	6.56	ND	7.44	21.38
TS-463	3.06	ND	4.18	ND	6.36	ND	6.75	15.86
TS-464	-	ND	3.34	ND	5.09	ND	5.41	20.62
TS-491	6.41	ND	10.23	ND	13.87	ND	14.48	11.02
TS-506	3.19	ND	5.32	ND	6.52	ND	7.13	18.96
TS-520	4.14	ND	7.66	ND	9.67	ND	10.48	15.64
Mean	3.98		6.33		8.43		8.70	18.75
S.Ed(±)	0.12		0.12		0.17		0.11	1.69
$CD_{t\,0.05}$	0.21		0.21		0.30		0.20	2.95
Correlation coefficient	ND		ND		ND		-0.67*	

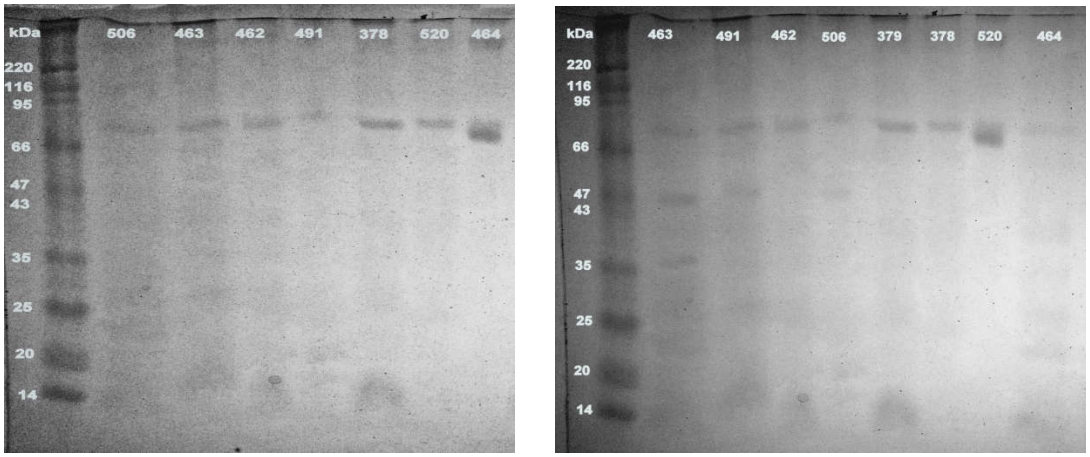
*: Statistically significant
ND: Not detected, - Not determined
A: 1st month after fruit development; B: 3rd month after fruit development; C: 5th month after fruit development; D: 8th month after fruit development



(A) 1st month after fruit development



(B) 3rd month after fruit development



(C) 5th month after fruit development (D) 8th month after fruit development

Fig.1- Profiles of tea seed proteins of different bi-clonal seed stocks

CONCLUSION

There was a gradual increase of soluble protein content with development of fruit. The correlation studies indicated that there was negative significant correlation (-0.67) between the oil content with respect to soluble protein content of different bi-clonal seed stocks. From SDS-PAGE (10% resolving gel), a common band of size 66 kDa was detected. In future, analysing the proteins of the tea seed stocks with more informative tools like 2-D electrophoresis and LC-MS/MS techniques may improve our understandings of protein profiles of oleaginous tea seeds.

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