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Studies on morphological characters of induced autotetraploid som [*Persea bombycina* (King ex. Hook f.)] Kost genotypes

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Abstract

Four autotetraploid som genotypes along with their respective diploid parents were studied for 24 qualitative and 24 quantitative characters to establish distinctness, uniformity and stability (DUS) of tetraploid genotypes. Among 24 qualitative traits, 14 traits were monomorphic, 7 were dimorphic and 3 were polymorphic indicating their potentiality for characterization of the genotypes. The expression of each character in all genotypes was similar for three consecutive years confirming the stability of traits. Leaf and floral traits were most reliable for identification of tetraploids. The four tetraploids were distinct from each other and also from their respective diploid parents. For further characterization and to meet DUS criteria for identification of som, study of some of the distinct morphological characters in coalition with molecular character analysis might be necessary.

Key words: Induced autotetraploid, morphological character, *Persea bombycina*, som

Introduction

Som (*Persea bombycina* Kost) is the principal host plant (family lauraceae) of muga silkworm (*Antherea assamensis* Helfer). It is a medium sized, perennial, evergreen, cross pollinated tree. In germplasm bank of Central Muga Eri Research and Training Institute (CMER&TI), Lahdoigarh, Jorhat, Assam, India, 51 genotypes of som are being maintained. Among these, four genotypes are tetraploids developed through polyploidization technique from diploid genotypes [1]. Accurate description of these tetraploid genotypes is needed for distinctness, uniformity and stability (DUS) testing. Establishing distinctness of a variety will not only help in Plant Variety Protection (PVP) legislation for new plant variety under the Protection of Plant Varieties and Farmers Rights Act, [2] but will also help in seed certification programme and identification of duplicates in germplasm collection. The tetraploids may also serve as a source of breeding materials for production of triploid som varieties. Though tetraploids were identified for their superiority in leaf yield, effective rate of rearing (ERR) and economic traits of cocoon [1], but they were not properly characterized morphologically due to lack of systematic studies with respect to morphological traits [3]. The morphometric studies are useful for identification of species [4, 5].

The present investigation was undertaken for morphological description of tetraploid som genotypes and their respective diploid parents and for distinguishing tetraploids som genotypes at different stages of development for future applications in selection and popularization of good varieties for wider commercial cultivation as well as breeding programme.

Material and methods

Four genotypes of som, *viz.*, PB003, PB004, PB005 and PB006 were taken from the field gene bank of Central Muga Eri Research & Training Institute, (CMER&TI) Lahdoigarh, Assam, India for studies. The apical buds were treated with 0.5% aqueous solution of colchicines using cotton swab. Concentration of colchicines solution was determined through initial screening experiments and it was found that 0.5% colchicines solution (aqueous) was most effective. Leaf tips of treated vegetative buds along with control were collected and pretreated with saturated solution of P-dichlorobenzene at 10-12^{o}C for 6-8 hours followed by fixation in 1:1 Propionic-ethanol for 24 hours. The leaf tips were subsequently hydrolyzed in 1N HCl and 2% propiono-orcein mixture (1:9) and kept for overnight. Standard squash technique was followed for the cytological study. Microphotograph was taken from temporary slides.

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Tetraploid plants were propagated through airlayering technique and accession number was given to tetraploids developed from PB003, PB004, PB005, and PB006 as PB009, PB010, PB011 and PB012, respectively. Four tetraploid genotypes i.e., PB009, PB010, PB011 and PB012 and their respective diploid parents PB003, PB004, PB005 and PB006 were taken for morphological studies. The selected genotypes were five years old, planted at 3x3 m spacing in three replication following standard package and practices [6]. The identification of genotypes was done using 24 qualitative and 24 quantitative characters as described in Table 1. For recording various observations on qualitative and quantitative characters, three plants per replication were used for period of three consecutive years.

Table 1. Morphological characters and their states

S.No.	Character	Characters state
Qualitative characters		
1	Growth nature	Bushy, upright, intermediate, spreading, drooping
2	Branching nature	straight, slightly curved, curved
3	Young shoot colour	Light green, green, purple, greenish purple
4	Mature shoot colour	Brown, gray, grayish green, greenish brown,
5	Phyllotaxy	Distichous (1/2), tristichous (1/3), pentastichous (2/5)
6	Lenticel density/cm^2	Sparse (<5), medium (5-9), dense (>10)
7	Lenticel shape	Round, elliptical, oval
8	Bud shape	Round, acute triangle, long triangle, spindle
9	Bud attachment	Slanting outward, adhering to branch, tilting to one side
10	Leaf type	Simple, compound
11	Leaf nature	Lobed, unlobed
12	Leaf shape	Elliptical- narrow elliptic (L:W=3:1), elliptic (2:1), wide elliptic (1.5:1); Obovate-oblaceolate (3:1), obovate (2:1)
13	Leaf apex	Acute, acuminate, attenuate
14	Leaf margin	Entire, repand
15	Leaf base	Acute, obtuse, cuneate
16	Leaf angle	Acute(<35°), semi erect (35°-75°), horizontal (>75°-90°)
17	Shape of the leaf scar	Circular, elliptical, triangle
18	Young leaf colour	Light green, green, greenish purple, purple
19	Mature leaf colour	Light green, green, dark green
20	Mature leaf surface	Smooth, smooth with glossiness
21	Petiole colour	Light green, green, light purple, purple
22	Vein colour	Light green, green, light purple, purple
23	Venation	Craspedodromous (simple, semicraspendodromous, mixed), Camptodromous (brochidodromous, eucamptodromous)
24	Veinlets	Simple, branched

Quantitative characters

1	Lamina length (L) (cm)	13	Pedicel length (mm)
2	Lamina width (W) (cm)	14	Number of petals /flower
3	L: W ratio	15	Number of stamen/flower
4	Petiole length (cm)	16	Number rudimentary stamen/flower

5	Petiole width (cm)	17	Stamen length (mm)
6	Protecting cover number of flowering bud	18	Anther length (mm)
7	Leaf thickness(µm)	19	Anther width (mm)
8	Number of stomata/field	20	Style length (mm)
9	Stomata size Lx W (µm)	21	Number of fruits/inflorescence
10	Inflorescence length (cm)	22	Fruit length (mm)
11	Inflorescence width (cm)	23	Fruit width (mm)
12	Number of flowers/inflorescence	24	Fruit weight (g)

Results and discussion

Qualitative traits of the genotypes (Table 1) were found to be stable and uniform for the period of three years. Stability of genotypes refers to production of a narrow range of phenotypes in different environment, indicating more effect of genotype than the environment. This is due to the fact that qualitative traits were produced by oligogenes. Such traits show high heritability and were easy to select. Hence, stability and uniformity are the two aspects emphasized by breeders for plant improvement programme.

The study of qualitative characters revealed that no single character but a set of characters together could classify the genotypes. Fourteen traits were found to be monomorphic, 7 were dimorphic and 3 were polymorphic indicating their potentiality for characterization of the genotypes (Table 2). The observations suggested variation among the tetraploids PB009, PB010, PB011 and PB012 and their respective diploid parents PB003, PB004, PB005 and PB006 for few characters like bud and leaf shape, leaf apex, branching nature, and young shoot colour though they showed similarity for other traits. The variation among the traits might be due to their differential genetic behaviours. In this study, both diploid and tetraploid were found to be of spreading type with straight branches except for PB009 whose branches were slightly curved. The tetraploids showed variation for young shoot colour among themselves but similarity with their respective diploid parents. PB003, PB009, PB005, PB011 had light green, PB004 and PB010 had green and PB006 and PB012 had light greenish purple young shoot. All the genotypes had brown mature shoot having pentastichous phyllotaxy and round shaped lenticels. The lenticels density of the tetraploids was found to be sparse whereas diploids had medium density.

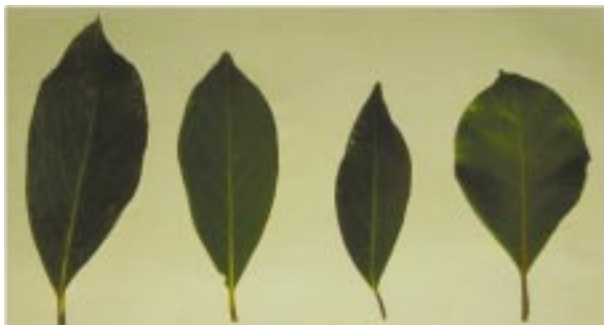
The bud morphological characters such as bud shape and bud attachment were easy to detect and could classify som varieties into few broad categories. The bud shape of PB009, PB010 and PB011 was same as their diploid parent's i.e. acute triangle, spindle and long triangle, respectively but PB009 had wider bud base. Bud shape of PB012 was obtuse triangle whereas its diploid parent had acute triangle shaped bud. Bud in all the genotypes was attached to the branch by slanting outward.

Leaf character of som is the most reliable trait for classification. Based on leaf shape, som genotypes were classified into different morphotypes [7, 8]. Though, in the present study all the studied genotypes had simple, unlobed leaf with entire margin, acute leaf base, semi erect leaf angle and circular leaf scar but they showed wide variation for leaf shape and leaf apex (Fig. 1). Thus, based on this trait plants could be grouped into distinct classes for useful varietal identification and genetic purity testing. The leaf shape of diploids PB003 and PB004 was oblaceolate, PB005 was narrow elliptical and that of PB006 was wide oblaceolate. After polyploidization the leaf shape of respective tetraploids were found to be wide oblaceolate (PB009, PB010), elliptical (PB011) and narrow obovate (PB012). The tetraploids PB009 and PB010 had acute leaf apex but their diploids parents had acuminate and attenuate leaf apex respectively. PB011 and PB012 leaf had same apex as their diploid parents i.e. attenuate and acuminate, respectively. Leaves were light green when young and changed to green in diploids and dark green in tetraploids when matured, though leaf surface remain smooth throughout their life in both diploids and tetraploids som. Petiole and vein colour of all the genotypes were light green except for PB012 which had light purple. Leaf of all the genotypes had brochidromous venation and branched veinlets.

The most important factor by which tetraploids can easily be differentiated from diploid was the cytological study. All the diploid som had 24 chromosomes whereas all the tetraploids had 48 chromosomes (Fig. 2).

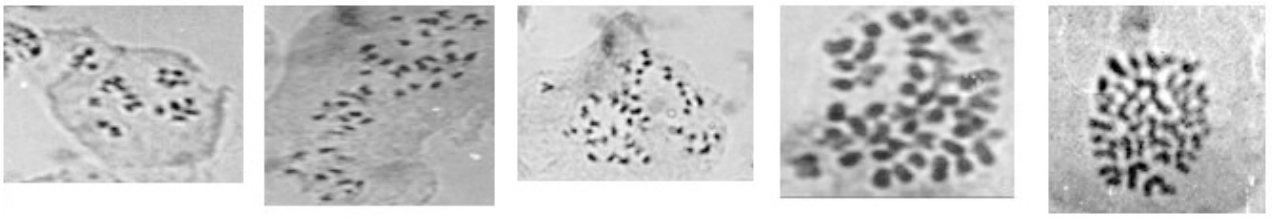
Table 2. Morphological (qualitative) characters of tetraploid som genotyoes

Characters	Diploid (2n=2x=24)				Tetraploid (2n=4x=48)			
	PB003	PB004	PB005	PB006	PB009	PB010	PB011	PB012
Growth nature	Spreading	Spreading	Spreading	Spreading	Spreading	Spreading	Spreading	Spreading
Branching nature	Straight	Straight	Straight	Straight	Slightly curved	Straight	Straight	Straight
Young shoot colour	Light green	Green	Light green	Light greenish purple	Light green	Green	Light green	Light greenish purple
Mature shoot colour	Brown	Brown	Brown	Brown	Brown	Brown	Brown	Brown
Phyllotaxy	Pentastichous	Pentastichous	Pentastichous	Pentastichous	Pentastichous	Pentastichous	Pentastichous	Pentastichous
Lenticels density/sq.cm	Medium	Medium	Medium	Medium	Sparse	Sparse	Sparse	Sparse
Lenticels shape	Round	Round	Round	Round	Round	Round	Round	Round
Bud shape	Long triangle	Acute triangle	Acute triangle	Acute triangle	Long triangle	Long triangle	Acute triangle	Long triangle
Bud attachment	Slanting outward	Slanting outward	Slanting outward	Slanting outward	Slanting outward	Slanting outward	Slanting outward	Slanting outward
Leaf type	Simple	Simple	Simple	Simple	Simple	Simple	Simple	Simple
Leaf nature	Unlobed	Unlobed	Unlobed	Unlobed	Unlobed	Unlobed	Unlobed	Unlobed
Leaf shape	Oblaceolate	Oblaceolate	Narrow elliptical	Wide oblaceolate	Oblaceolate	Wide oblaceolate	Elliptical	Narrow obovate
Leaf apex	Acuminate	Attenuate	Attenuate	Acuminate	Acute	Acute	Attenuate	Acuminate
Leaf margin	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Leaf base	Acute	Acute	Acute	Acute	Acute	Acute	Acute	Acute
Leaf angle	Semi erect	Semi erect	Semi erect	Semi erect	Semi erect	Erect	Erect	Erect
Shape of the leaf scar	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Young leaf colour	Light green	Light green	Light green	Light green	Light green	Light green	Light green	Light green
Mature leaf colour	Green	Green	Green	Green	Dark green	Dark green	Dark green	Dark green
Mature leaf surface	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Petiole colour	Light green	Light green	Light green	Light green	Light green	Light green	Light green	Light purple
Vein colour	Light green	Light green	Light green	Light green	Light green	Light green	Light green	Light purple
Venation	Brochido-dromous	Brochido-dromous	Brochido-dromous	Brochido-dromous	Brochido-dromous	Brochido-dromous	Brochido-dromous	Brochido-dromous
Veinlets	Branched	Branched	Branched	Branched	Branched	Branched	Branched	Branched



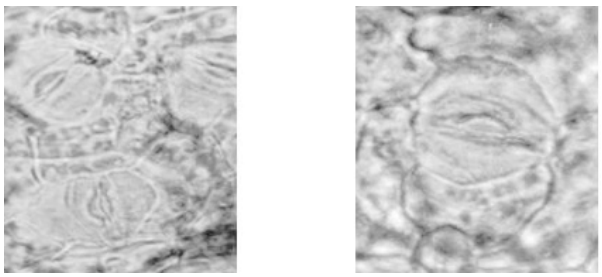
PB 009 PB 010 PB 011 PB 012

Fig. 1. Leaf shape of tetraploids



Diploid (2n=24) PB 009 PB 010 PB 011 PB 012

Fig. 2. Mitotic chromosomes of diploid and tetraploid genotypes



Diploid Tetraploid

Fig. 3. Stomata

PB 009 PB 010

PB 011 PB 012

Fig. 4. Inflorescence of trtraploid plants

Fig. 5. Mature tetraploid flower

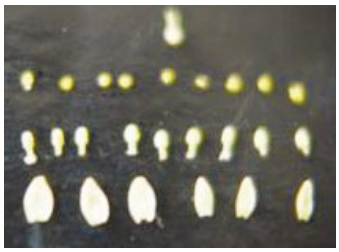


Fig. 6. Flower parts of tetraploid (PB 009)

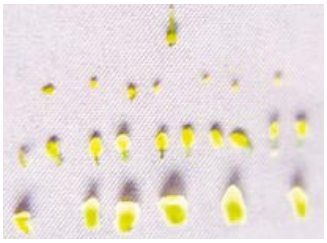


Fig. 7. Flower parts of tetraploid (PB 012)

Quantitative traits revealed wide variation both between and within diploid and tetraploid genotypes (Table 3). Based on leaf morphology and floral biology 8 distinct som morphotypes were identified and isolated (9) indicating these traits to be crucial for differentiation. Hence, for the present study detailed observations of leaf and floral trait were carried out.

Length, width and length: width ratio indicated leaf of all the tetraploid genotypes to be larger and wider than their respective diploid parent's leaf (Fig. 1). As muga host plant leaf is an important economic trait of som, thus tetraploids provide scope for breeding for higher yield by selecting genotypes with larger and wider leaf. Tetraploids gave higher value for number of protecting cover of flowering bud, thickness of leaf, petiole length and width but lower stomata number than diploids which might be due to larger stomata size in tetraploids (Fig. 3, Table 3).

The tetraploids showed large variation also for floral character (Table 3). Som inflorescence is a panicle having hermaphrodite flower. In this study, inflorescence length and width and number of flowers/inflorescence (Fig. 4) were found to be higher, though, pedicel length was found to be lower in tetraploids than diploid parents. PB009 and PB011 had same number of stamen/flower [9] as the diploid flower had but PB010 and PB012 had 10 number of stamen/flower. Tetraploid flowers had only 7-9 number of rudimentary stamen though diploid flower showed 9 number of rudimentary stamen (Figs. 5, 6 & 7). Number of petals/flower was same for all the genotypes. After polyploidisation the stamen length of tetraploids was reduced but anther length and width and style length were increased. Tetraploids produced larger and heavier fruits than diploid parents.

Thus, such studies not only characterize a species to establish their identity but also provide new bases

Table 3. Morphological characters (quantitative) of tetraploid som genotypes

Characters	Diploid Range	Tetraploid Range	Mean	SD
Lamina length (cm)	10.27-12.48	11.06-13.92	11.66	±1.18
Lamina width (cm)	3.39-4.1	4.54-6.14	4.57	±0.93
Lamina length **:** width ratio	2.66-3.17	1.80-2.85	2.62	±0.46
Petiole length (cm)	1.12-2.25	1.35-1.8	1.52	±0.38
Petiole width (cm)	0.2-1.28	0.25-0.3	0.25	±0.05
No. of protecting cover of flowering bud	11.8-15.4	24.8-29.4	20.63	±7.75
Thickness of leaf (µm)	265.32-288.42	291.72-327.36	289.49	±20.68
No. of stomata/field	24-30	18-20	22.75	±4.53
Stomata size L x W (µm)	77.7-96.52	114.57-160.87	113.07	±33.23
Inflorescence length (cm)	4.2-5.5	5.2-8.9	5.53	±1.46
Inflorescence width (cm)	7.2-10.26	7.24-13.88	9.12	±2.44
No. of flower/ Inflorescence	48-77.6	48.6-87.4	63.01	±14.86
Pedicel length (mm)	2.84-4.54	2.6-4.13	3.42	±0.75
No. of petal /flower	6	6	6	0
No. of stamen /flower	9	9-10	9.25	±0.54
No. of rudimentary stamen	9	7-9	8	±1.07
Stamen length (mm)	1.35-1.52	1.11-1.4	1.33	±0.15
Anther length (mm)	1.26-1.37	1.42-1.56	1.39	±0.12
Anther width (mm)	0.6-0.8	0.9-1.0	0.83	±0.15
Style length (mm)	1.33-2.96	1.7-2.6	2.01	±0.53
No. of fruit/ inflorescence	3.8-8.6	2-4	4.2	±1.96
Fruit length (mm)	8.18-8.5	8.4-10.16	8.76	±0.68
Fruit width (mm)	7.06-8.12	7.96-8.64	8.18	±0.58
Fruit weight (g)	0.29-0.38	0.33-0.59	0.38	±0.09

for selection and conservation of the species and helps in realizing the genetic potential of the genotypes. However, further investigations particularly molecular characterizations are required to meet the DUS criteria for varietal protection.

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