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RESEARCH ARTICLE

FREQUENCY DISTRIBUTION OF QUALITATIVE AND QUANTITATIVE TRAITS IN FORAGE MAIZE (*ZEA MAYS*)

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SUMMARY Frequency distribution of qualitative and quantitative traits was worked out in 101 forage maize accessions along with known forage maize variety, African Tall for various fodder and kernel yield traits. For qualitative traits, 6 major groups showed significant variation in kernel colour and for quantitative traits, 5 major groups of accessions were formed on the basis of mean performance of each accession. Accessions under group V showed the highest mean values for all the traits and maximum values for yield. African Tall showed promising results for most of the characters and accessions of late flowering type have been found to be better in fodder and kernel production.

Keywords: Forage maize, *Zea mays*, frequency distribution, quali-quantitative traits.

INTRODUCTION

The two basic requirements of plant breeding are, the presence of genetic variation and exploitation of this variation through selection. Vavilov (1951) stressed the importance of variation for rapid improvement. Selection involves the identification and isolation of desirable plants from a variable population. The selection of plants from a population is always based on their appearance which has both heritable and non-heritable components. The heritable component is due to the genes present and non-heritable component is resulted from the effects of environment. Some characters are little affected by either genes or the environment. Such characters are generally governed by one or few genes with large, easily detectable effects known as oligogenes. The characters produced by oligogenes show distinct classes and are known as qualitative characters or oligogenic traits. On the other hand, the development of many characters is highly affected by the genetic factors and, more particularly by the environment. These characters are governed by several genes with small individual effects and are known as polygenes. The characters produced by polygenes are referred to as quantitative characters, because they do not show clear-cut classes and have to be studied by measurements.

From tassel to root, the maize plant is valuable. The stalk, leaves, silk, cob and kernels all have a commercial value. It surpasses all other crops used as fodder in dry matter production and digestible nutrient per hectare. Most of the

research work has been carried out in India and abroad on the grain aspect of maize crop. Hence, no attempt has been made so far in India to classify genetic material of forage maize. Although maize has been used for a long time as a dual purpose crop, the forage based collection, evaluation and documentation of maize germplasm have not been done as yet. Therefore, it was felt that there is a need to make a systematic attempt for collection, assessment and categorization of forage maize for forage as well as kernel yield and its related trait. The present study is an attempt made in that direction.

MATERIALS AND METHODS

A collection comprising 100 accessions of forage maize (*Zea mays*) was made from Madhya Pradesh, Rajasthan and Uttar Pradesh. These accessions were evaluated with control variety of forage maize (African Tall) in a randomized block design (RBD) with 3 replications at Central Research Farm, Indian Grassland and Fodder Research Institute, Jhansi during 2001-2003. Each accession was evaluated in plot of 2 rows of 4 m length at 0.40 m apart. Observations on various qualitative and quantitative parameters contributing to fodder yield potential were recorded at 50% silking stage of the accession, whereas, the data on kernel yield and its attributes were recorded at 80% maturity of the line. The accessions were grouped into 6 for qualitative and 5 for quantitative frequency classes on the basis of observation and mean performance of each accession. Means were computed by dividing the sum of all the observations in a sample by their numbers.

OBSERVATIONS

Distribution of forage- and kernel-based qualitative traits

The frequency distribution of qualitative characters studied was classified into 6 major groups for 101 accessions (Table 1). Among 101 accessions studied, 85 accessions had dark green leaf blades while the remaining 16 accessions showed light green leaf blades. Regarding sheath colour variability, 69 accessions had dark green sheath and 32 were having light green sheath. However, no colour variation was noticed in midrib colour as all accessions have white midrib. Similar to the colour variability of leaf in respect of blade and sheath, stem colour also varies. Whereas the stems of 34 accessions are dark green, 67 accessions showed light green stem.

Another qualitative trait which shows a high degree of variability is kernel which shows variation in colour, arrangement, shape and size. The kernel colour differs greatly with accessions ranging from white, variegated and brown to yellow. Twentytwo accessions have dark yellow kernels, 49 of light yellow, 14 of white, 8 of variegated, 7 of yellow and only one accession had brown kernels. The kernel row arrangement also differs greatly ranging from regular, straight and irregular to spiral. Regular arrangement has been observed in 53 accessions, and 27 accessions have straight, and 15 accessions have irregular arrangement and only 6 accessions showed spiral row arrangement. Kernel shape showed variabi-lity with pointed, indented and round to almost shrunken. Eightythree accessions had shrunken kernels, 15 of round, 2 of indented

while only one accession showed pointed kernels. Seventeen accessions showed smaller kernels, 53 of medium size and 31 accessions showed bold kernels. The shape of cob varies. Fifty three accessions had cylindrical cob shape while 48 accessions showed conical shape (Table 1).

TABLE 1: Frequency distribution of qualitative traits in maize.

Character	Group - I	Group - II	Group - III	Group - IV	Group - V	Group - VI
Leaf blade colour	Dark green (85)	Light green (16)	–	–	–	–
Sheath colour	Dark green (69)	Light green (32)	–	–	–	–
Midrib colour	White (101)	–	–	–	–	–
Stem colour	Dark green (34)	Light green (67)	–	–	–	–
Kernel colour	Dark yellow (22)	Light yellow (49)	White (14)	Variegated (8)	Brown (1)	Yellow (7)
Kernel row arrangement	Regular (53)	Straight (27)	Irregular (15)	Spiral (6)	–	–
Kernel shape	Shrunken (83)	Round (15)	Indented (2)	Pointed (1)	–	–
Kernel size	Small (17)	Medium (53)	Bold (31)	–	–	–
Cob shape	Cylindrical (53)	Conical (48)	–	–	–	–

Numbers in parenthesis indicate numbers of accessions.

Distribution of forage- and kernel-based quantitative traits

On the basis of mean performance of each character, 5 major groups were formed for fodder and kernel yield traits separately (Tables 2, 3). This grouping indicated more number of accessions in groups II and III. For days to 50% silking, 43 accessions showed early growth type under group–I while African Tall comes under late for this trait. For plant height (56), number of leaves per plant (60) and stem girth (51) maximum accessions were reported in group–II while accessions under group–V were having only one genotype for this trait with maximum mean value namely, African Tall. For leaf blade length (54), sheath length (49) and leaf width (49) maximum accessions were reported in group–III. For green fodder yield/plant (g), maximum accessions were placed in group–II having 76 accessions and group–V had only one genotype, African Tall having maximum green fodder yield/plant. For dry fodder yield, group–III was

TABLE 2: Frequency distribution off odder yielding traits in maize.

Character	Group – I	Group - II	Group – III	Group – IV	Group - V
Days to 50% silking	43 –46.24 (43)	46.25–49.49 (24)	49.50 –52.74 (27)	52.75 –55.99 (06)	56.00–59.24 (01)
Plant height (cm)	148.44–172.53 (14)	172.54–196.63 (56)	196.64–220.73 (27)	220.74–224.83 (03)	244.84–268.93 (01)
No. of leaves/ plant	9.81–11.34 (25)	11.35–12.88 (60)	12.89–14.42 (15)	14.43–15.96 (-)	15.97–17.44 (01)
Leaf blade length (cm)	65.67–73.50 (02)	73.51–81.34 (18)	81.35–89.18 (54)	89.19–97.02 (21)	97.03–104.81 (06)
Sheath length (cm)	13.09 –14.58 (03)	14.59–16.08 (27)	16.09–17.58 (49)	17.59–19.08 (19)	19.09–20.54 (03)
Leaf width (cm)	6.44–7.21 (04)	7.22–7.99 (25)	8.0–8.77 (49)	8.78–9.55 (18)	9.56–10.33 (05)
Stem girth (cm)	1.54 –1.82 (11)	1.83–2.11 (51)	2.12–2.40 (38)	2.41–2.69 (-)	2.70–2.94 (01)
Green fodder yield/plant (g)	208.83–447.9 (14)	448–687.07 (76)	687.08–926.15 (10)	926.16–1165.23 (-)	1165.24–1404.17 (01)
Dry fodder yield/plant (g)	41.57–67.31 (06)	67.32–93.06 (38)	93.07–118.81 (52)	118.82–144.56 (04)	144.57–170.29 (01)
Leaf - stem ratio	0.23–0.30 (08)	0.31–0.98 (38)	0.39–0.46 (44)	0.47–0.54 (08)	0.55–0.62 (03)
Crude protein (%)	8.66–9.37 (02)	9.38 – 10.09 (06)	10.10–10.81 (48)	10.82 –11.53 (34)	11.54–12.24 (11)

Numbers in parenthesis indicate numbers of accessions.

the largest having 52 accessions and group–V were having only one genotype namely, African Tall. For leaf-stem ratio, group–III was largest with 44 accessions and the maximum leafy part was reported for group–V having three accessions namely, IC-335068, IC-334889, and IC-335131 from Uttar Pradesh, Rajasthan and Uttar Pradesh respectively. With regards the quality aspect, mainly for crude protein content group–III was largest with 48 accessions and 11 accessions were rich in crude protein content.

For days to maturity, maximum accessions were found in group–I having 49 accessions and group–V had only one genotype namely, African Tall which was late in maturity (Table 3). Considering the mean performance of cob length (38), cob width (37), number of kernel rows (41), number of kernels per row (31) and shank diameter (39) showed maximum accessions in group–III while for kernel length (41) and kernel width (42) maximum number of accessions were placed in group–II. For test weight, group–III was

TABLE 3: Frequency distribution of seed yielding traits in maize.

Character	Group – I	Group - II	Group – III	Group – IV	Group - V
Days to maturity	70.00 – 79.86 (49)	79.87 – 89.73 (39)	89.74 – 99.66 (12)	99.67 – 109.56 (-)	109.57 –119.33 (01)
Cob length (cm)	11.87 – 13.76 (17)	13.77 – 15.66 (34)	15.67 – 17.56 (38)	17.57 – 19.46 (10)	19.47 – 21.34 (02)
Cob width (cm)	3.06 – 3.22 (09)	3.23 – 3.39 (23)	3.40 – 3.56 (37)	3.57 – 3.73 (24)	3.74 – 3.90 (07)
No. of kernel rows	10.00 – 10.95 (03)	10.96 – 11.91 (29)	11.92 – 12.87 (41)	12.88 – 13.83 (23)	13.84 – 14.74 (05)
No. of kernels/ row	19.59 – 23.75 (10)	23.76 – 27.92 (21)	27.93 – 32.09 (31)	32.10 – 36.26 (22)	36.27 – 40.42 (17)
Shank diameter (cm)	0.98 – 1.11 (13)	1.12 – 1.25 (37)	1.26 – 1.39 (39)	1.40 – 1.53 (10)	1.54 – 1.62 (02)
Kernel length (cm)	0.75 - 0.80 (11)	0.81 – 0.86 (41)	0.87- 0.92 (33)	0.93 – 0.98 (13)	0.99 – 1.02 (03)
Kernel width (cm)	0.74 – 0.79 25	0.80 – 0.85 42	0.86 – 0.91 28	0.92 – 0.97 05	0.98 – 1.03 01
Test weight (g)	14.53 – 16.77 (08)	16.78 – 19.02 (30)	19.03 – 21.27 (39)	21.28 – 23.52 (18)	23.53 – 25.73 (06)
Kernel yield/ plant (g)	126.21 – 153.29 (13)	153.30 – 190.38 (42)	180.39 –207.47 (17)	207.48 – 234.56 (22)	234.57 – 261.61 (07)

Number in parenthesis indicate number of accessions.

largest having 39 accessions and maximum values for this trait were reported in group–V having only 5 accessions with African Tall namely, IC-334853, IC-334869, IC-334932, IC-335024 and IC-334954. These accessions were explored from Rajasthan except IC-335024 collected from Uttar Pradesh. For seed yield/ plant, group–II was largest with 42 accessions and maximum seed yield was reported for group–V having 6 accessions with African Tall namely, IC-335024, IC-335116, IC-335094, IC-334974, IC-335122, and IC-334999 which were collected from Uttar Pradesh.

Considering mean performance of the accessions, the accessions, IC-335056 and IC-334973 were observed to be earliest for days to 50% silking as they flowered in 43 and 48 d respectively, whereas, the genotypes, African Tall and IC-334833 were considered late in this trait as they flowered in 59 and 54 d respectively (Table 4). For plant height, African Tall and IC-334855 were found to be taller as compared to others. The accessions identified as dwarf in plant height are, IC-335060 and IC-335056. The genotypes, producing maximum number of leaves are, African Tall and IC-335035. Maximum green fodder yield was observed in African Tall and IC-334846 whereas maximum dry fodder yield was recorded for African Tall and IC-334833.

TABLE 4: Desirable accessions in maize for various fodder traits.

Character		Accession
Days to 50% silking	Early	IC- 335056, 334973, 334999, 335000, 335068, 335086, 334915, 335062, 335111 and 335184.
	Late	African Tall, CI- 334833, 334853, 334863, 334836, 334945, 334834, 334838, 334942 and 334943.
Plant height	Dwarf	IC- 335060, 335056, 335086, 335069, 335062, 335068, 335079, 335082, 335116 and 334821.
	Tall	African Tall, IC- 334855, 334830, 334846, 334834, 334833, 335035, 334942, 334838 and 335032.
No. ofl eaves/ plant		African Tall, IC- 335035, 334943, 334864, 334842, 335041, 334876, 334855, 334942 and 334841.
Leaf blade length		African Tall, IC- 334999, 334855, 334837, 334833, 334872, 334846, 335053, 335009 and 335043.
Sheath length		African Tall, IC- 334833, 334846, 334879, 334855, 334830, 334872, 335035, 335089 and 335148.
Leaf width		IC- 334932, African Tall, IC- 334846, 334838, 334841, 334842, 334837, 334833, 334834 and 335128.
Stem girth		African Tall, IC- 334848, 334833, 334834, 334846, 334945, 334838, 334842, 334830 and 335041.
Green fodder yield/ plant		African Tall, IC- 334846, 335053, 334833, 334855, 334872, 335017, 334943, 334830 and 334841.
Dry fodder yield/ plant		African Tall, IC- 334833, 334846, 334834, 334855, 334942, 334830, 335000, 335053 and 334842.
Leaf - stem ratio		IC- 335068, 334889, 335031, 335110, 335144, 335079, 334836, 335035, 335069 and 335120.
Crude protein		IC- 334841, 334920, 335148, 334904, 334889, 335103, 334880, 335051, 335060 and 334848.

Being a cereal crop, maize is not rich in protein content. However, some accessions like IC-334841 and IC-334920 were identified to be very promising as they contain about 11–12.24% crude protein in leaf and stem parts whereas African Tall had very low crude protein content amongst all accessions. These results were supported by the earlier findings of Singh & Katiyar (1999) and Samanta et al. (2003).

Among the seed yield traits, the germplasm lines like IC- 335111 and IC-335069 were found to be early maturing type whereas, African Tall and IC-334904 were found late in maturity (Table 5). Maximum cob length was recorded for African Tall and IC-335024. Maximum cob width was reported for IC-334932 and IC-334877. IC-334942 and IC-334947 were rich in number of kernel rows and maximum kernels per row were

TABLE 5: Desirable accessions in maize for various seed traits.

Character		Accession
Days to maturity	Early	IC- 335111, 335069, 335056, 334973, 335068, 335086, 335062, 335060, 334974, and 335098.
	Late	African tall IC- 334904, 334945, 334943, 334942, 334834, 334954, 334853, 334867 and 334864.
Cob length		African tall, IC- 335024, 335996, 335110, 335117, 335045, 335009, 335050, 335158 and 334999.
Cob width		IC- 334932, 334877, 335032, 335024, 334999, 334944, 335009, 334842 and African tall.
No. of kernel rows		IC- 334942, 334947, 334942, 335158, 334848, 335060, 335079, 335068, 335050 and 335041.
No. of kernels/ row		IC- 335094, 335120, 335158, African tall, 335117, 335035, 335109, 335173, 335027 and 335111.
Shank diameter		African tall, IC- 334996, 335024, 335116, 335025, 334974, 335043, 335131, 335045 and 335048.
Kernel length		IC- 334954, 334853, 334932, 334943, 334846, 334869, 334825, 334842, 335017and 334841.
Kernel width		IC- 334869, 334846, 334932, 334838, 334954, 334853, 335148, 334836, 335092 and 334867.
Test weight		African tall, IC- 334853, 334869, 334932, 335024, 334954, 334871, 334841, 334863 and 334884.
Kernel yield/ plant		African tall, IC- 335024, 335116, 335094, 33334974, 335022, 334999, 334853, 335148 and 334877.

observed in IC-335094 and IC-335120. The germplasm lines with big shank size were, African Tall and IC-334996. Accessions IC-334954 and IC-334853 were having big kernel length and IC-334869 and IC-334846 were found thick in kernel width. Maximum test weight was recorded for African Tall and IC-334853. African Tall also showed maximum seed yield/plant followed by IC-335024. These results are in conformity with those of Chen et al. (1996) Katiyar et al. (2001) and Turgut et al. (1995) .

DISCUSSION

The present investigation revealed considerable genetic variation for different fodder and kernel yield traits in different environment as well as over the environment. Among the quality traits maximum accessions were showing dark green leaf blade and leaf sheath and light green stem colour were reported. Among the kernel based quality traits maximum accessions were showing light yellow kernels and regular kernel row arrangement with cylindrical cob. Medium size

and shrunken kernels were also found in large number of accessions. These findings are in consonance with Katiyar et al. (2001).

Study showed that all the lines possessed lesser green fodder yield as compared to African Tall. As regard dry fodder yield, African Tall recorded quite higher yield as compared to other accessions and accessions with high dry fodder yield were also late in flowering. Malaviya et al. (2002) have also reported similar results. Gupta et al. (1984) recommended that fodder variety should be early growth and late flowering type as late flowering types have been observed to be better in fodder production. Srivas et al. (2012) concluded that fodder yield might be a good indicator for kernel yield for dual purpose variety in maize and could be developed by selecting plants with more plant height, number of leaves per plant, stem girth, days to maturation, test weight and kernel length alone or in combination.

Accessions of group–V were having maximum mean value regarding yield and its related traits and the maximum mean values recorded for accessions explored from Rajasthan and Uttar Pradesh. The grouping of accessions collected from different geographical regions can be useful for future breeding programme by selecting desirable traits. Based on nonhierarchical Euclidean cluster analysis, forage maize accessions grouped into eight clusters and revealed that with high and low cluster mean for majority of characters will help to improve fodder and kernel yield as well as quality in forage maize (Srivas et al. 2020). Li et al. (2004) have made a core collection of maize landraces and divided into subgroups in terms of plant height, number of leaves, ear length, ear width, kernel colour, number of rows and 1000-kernel weight. Singh et al. (2005) have also made core collections of forage maize from different geographical regions of India preserved in National Gene Bank (NBPGR, New Delhi, India). The accessions were evaluated and grouped for various fodder and kernel traits.

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RESEARCH ARTICLE

CHROMOSOME STUDIES ON FOUR SPECIES OF *APHIS* INFESTING FOOD CROPS

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SUMMARY Karyotypic studies on four species of genus *Aphis* that include *A. citricola* van der Goot infesting *Amaranthus caudatus*, *A. craccivora* Koch infesting *Vigna mungo*, *A. fabae* Scopoli infesting *Zea mays*, and *A. glycines* Matsumura infesting *Glycine max* were carried out. The diploid chromosome number in all these species was found to be 8. The chromosomes are holocentric. For karyotype analysis, chromosomes were measured at metaphase stage from ten selected plates. The total complement length and relative lengths were calculated for each species. Idiograms of these species showed a gradual decrease in the length of chromosome pairs.

Keywords: Karyotypes, *Aphis*, *Amaranthus*, *Vigna*, *Glycine*, *Zea,* total complement length.

INTRODUCTION

There are around 5000 known aphid species (Hemiptera: Aphididae) the world over. Of these, 450 species have been recorded from crop plants (Blackman & Eastop 2017). About 100 of these aphid species are of significant agricultural importance as they have infested the agricultural crops affecting their yield to a significant level (Van Emden & Harrington 2007).

Aphis is the most diverse and largest genus belonging to the family Aphididae. It comprises nearly 500 species (Eastop & Hille Ris Lambers 1976). Legumes have played a key role in the traditional diets of people throughout the world, and are an excellent source of protein, dietary fiber, starch, micronutrients, and phytochemicals with a low fat content (Pugalenthi & Vadivel 2008). However, these crops are subject to various pest outbreaks, reducing their yield. Aphids are one among the main pests of these agricultural crops in temperate regions (Dedryver et al. 2010).

Aphids have complex life cycles involving thelytokous parthenogenesis, polyphenism, viviparity, telescoping of generations and host alternation. Chromosomes of aphids are holocentric (Blackman 1980). They are characterized by a rapid karyotype evolution (Bures et al. 2013) which make them an interesting group to be studied cytogenetically. Keeping this in view, the present investigation was undertaken to study the chromosomes of commonly occurring aphid species, *A. citricola*, *A. craccivora*, *A. fabae* and *A. glycines* infesting legumes and cereals.

MATERIALS AND METHODS

For the chromosome studies, aphids were collected from leaves, flowers and pods of different crop plants from Mandal village of Shimla hills (altitude 1450 m above sea level). The infested plant parts along with aphids were brought to laboratory for further studies.

The somatic embryonic tissues were dissected out from apterous parthenogenetic females by puncturing the posterior end of the abdomen. Only the young embryos were used for chromosome studies, as cells in these embryos were mostly in divisional stages. Then, embryos were pretreated in 0.7% sodium citrate solution for 30 min. The pretreated embryos were fixed in 1:3 acetic-ethanol solution for about 15–20 min at room temperature. After fixation, embryos were squashed on a glass slide in a drop of 45% acetic acid for 3–5 min and stained with 2% Giemsa for about 25–30 min followed by mounting in DPX. The slides were observed under a research microscope and photomicrographs were taken. Well spread metaphase plates were selected for chromosomal measurements. Actual lengths of chromosomes were measured using ocular micrometer. The total complement length (TLC) and relative lengths were calculated. Chromosome pairs were arranged in decreasing order of their lengths to prepare the karyotype of each species.

OBSERVATIONS

In the present study, chromosomes of four species of *Aphis* namely, *A. citricola,* infesting *Amaranthus caudatus A. craccivora,* infesting *Vigna mungo, A. fabae* infesting *Zea mays* and *A. glycines* infesting *Glycine max* have been studied.

A. citricola

This species has diploid chromosome number of 8 (Figs 1, 2). The length of chromosomes ranged from 1.72 µm to 4.26 µm. TCL was 23.82 µm. The relative length of chromosomes ranged from 7.27 to 18.06. The somatic complement consists of 4 pairs of chromosomes showing gradual decrease in their lengths (Fig. 3).

A. craccivora

This species has diploid chromosome number of 8 (Figs 4, 5). The length of chromosomes ranged from 2.31 µm to 3.74 µm. TCL was 23.95 µm. The relative length of chromosomes ranged from 8.72 to 14.12. The somatic complement consists of 4 pairs of chromosomes showing gradual decrease in their lengths (Fig. 6).

A. fabae

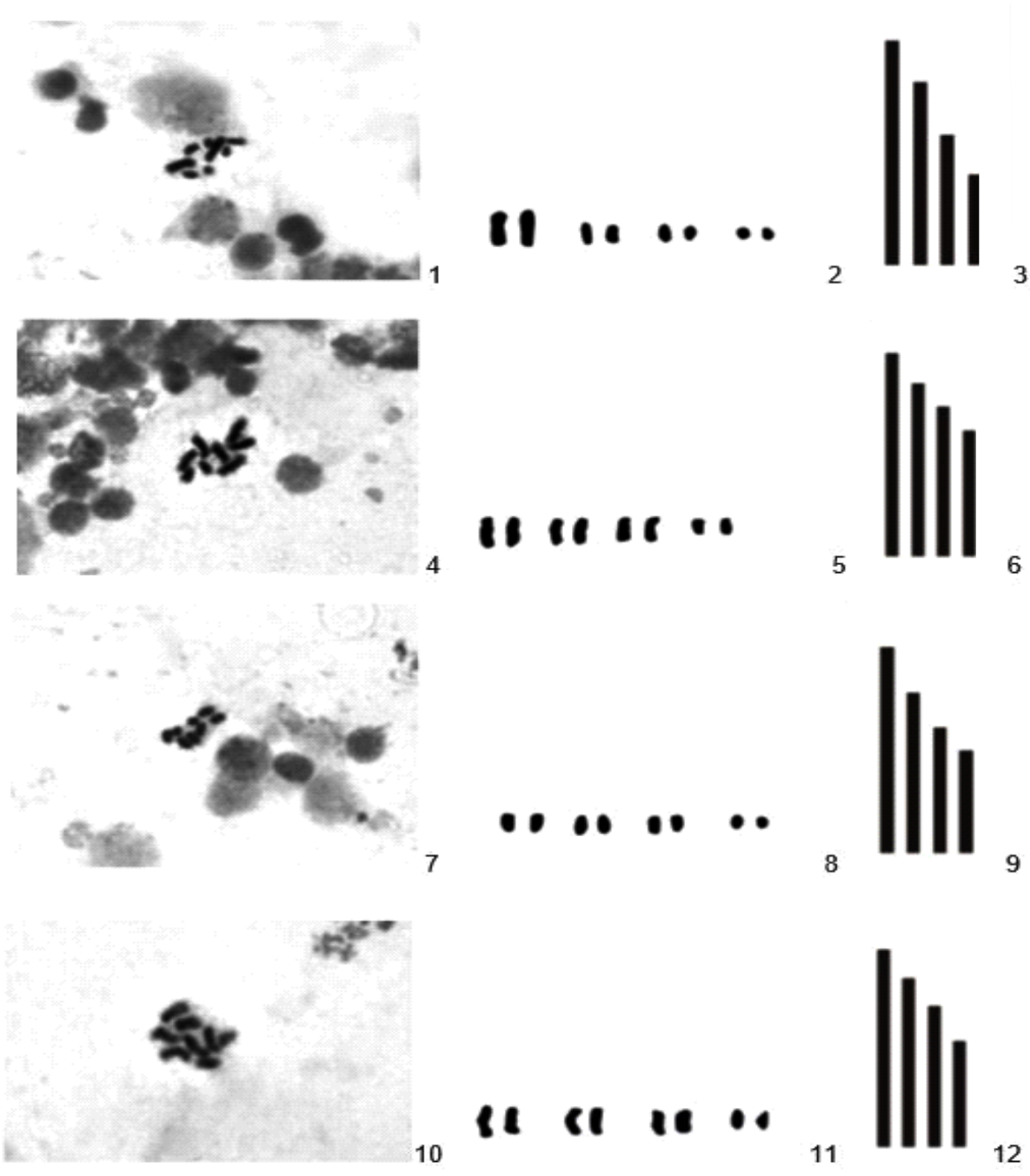
This species has diploid chromosome number of 8 (Figs 7, 8). The length of chromosomes ranged from 1.27 µm to 2.56 µm. TCL was 14.73 µm. The relative length of chromosomes ranged from 8.66 to 17.44. The somatic complement consists of 4 pairs of chromosomes showing gradual decrease in their lengths (Fig. 9).

A. glycines

This species has diploid chromosome number of 8 (Figs 10, 11). The length of chromosomes ranged from 1.73 µm to 3.24 µm. TCL was 20.09 µm. The relative length of chromosomes ranged from 8.22 to 15.34. The somatic complement consists of 4 pairs of chromosomes showing gradual decrease in their lengths (Fig. 12).

DISCUSSION

So far, about 80 species of *Aphis* have been



Figs 1–12: Karyotypes of aphids. 1–3. *A. citricola* 1. Somatic chromosomes 2. Karyotype. 3. Idiogram. 4–6. *A. craccivora.* 4. Somatic chromosomes. 5. Karyotype. 6. Idiogram. 7–9. *A. fabae*, 7. Somatic chromosomes. 8. Karyotype. 9. Idiogram. 10–12. *A. glycines*, 10. Somatic chromosomes. 11. Karyotype. 12. Idiogram.

investigated cytologically (Gavrilov-Zimin et al. 2015). All 4 species of *Aphis* included in the present investigation reveal the diploid chromosome number of 8.

A. citricola commonly known as green citrus aphid is considered as the most harmful pest of citrus trees. The diploid chromosome number of 8 reported here is in conformity with the earlier reports (Sun & Robinson 1966, Kar & Khuda-Bukhsh 1989, Dutta & Gautam 1993, Gautam & Dhatwalia 2003).

A. craccivora is a polyphagous aphid and is a major pest of legume crops. The diploid chromosome number of 8 reported here for this species was reported earlier by many workers from different host plants such as *Vigna catjung, Lathyrus sativus* (Kurl 1978), *Cassia fistula* (Kulkarni & Kacker 1979), *Dolichus biflorus, Ageratum conyzoides* (Kurl & Chauhan 1986), *Citrus* sp. (Dutta & Gautam 1993), *Taraxacum officinale, Minuartia micrantha* and *Trifolium ambigum* (Bakhtadze et al. 2010).

A. fabae is a common polyphagous and heteroecious pest infesting a wide range of secondary host plants. Aphids collected here from *Zea mays* has diploid chromosome number of 8, that is in conformity with the earlier reports of Colling (1955), Kuznetsova & Gandrabur (1991), Dutta & Gautam (1993), Blackman & Spence (1996) and Jangral et al. (2014).

Aphis glycines is one of the most serious pests of soybean worldwide. The diploid chromosome number of 8 reported in the present study is in conformity with the earlier reports of Kar & Khuda-Bukhsh (1991), Gautam & Dhatwalia (2003) and Khagta & Gautam (2016).

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RESEARCH ARTICLE

OPTIMIZATION OF CULTURE PARAMETERS FOR PRODUCTION OF CARBOXYMETHYL CELLULASE FROM *COLLETOTRICHUM GLOEOSPORIOIDES,* AN ORCHID ENDOPHYTIC FUNGUS

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SUMMARY : The present work was undertaken to screen and optimize culture conditions to obtain maximum carboxymethyl cellulase (CMCase) production from *Colletotrichum gloeosporioides*, an endophytic fungus associated with epiphytic orchid, *Cymbidium aloifolium*. The influence of different culture parameters like temperature, pH, carbon and nitrogen sources were determined on production of CMCase by the fungus. The highest enzyme activity of 0.2 ± 0.012 U/ml was at 30^0 C, pH 5 (0.52 ± 0.011 U/ml), carboxymethyl cellulose (CMC) as the carbon source (0.2 ± 0.023 U/ml) and yeast extract as nitrogen source (0.46 ± 0.012 U/ml).

Key words: *Colletotrichum gloeosporioides*, fungus**,** cellulase, enzyme activity.

INTRODUCTION

The endophytic fungi are reported to produce extracellular enzymes which help them to hydrolyze and absorb nutrition from its surroundings and also associated host plant. The extracellular enzymes produced by endophytic fungi degrade lignocellulosic fibers; hydrolytic enzymes like xylanase and cellulase degrade polysaccharides; oxidative lignolytic system produces laccase, ligninase and peroxidase which degrade lignin and open the phenyl ring system. The enzyme production and regulation by endophytic fungi could be owed to their genetic recombination with the host which has progressed with evolution. During the past few years, many researchers have attempted to catalogue the enzymes produced by endophytic fungi however, it has been barely exploited for industrial concern (Correa et al. 2014).

Cymbidium aloifolium is an epiphytic orchid growing at the height of 200–300 m on the trees. The epiphytic orchids utilize nutrients from host trees on which they are growing. The endophytic fungi associated with these orchids produce some extracellular enzymes which enable them to solubilize complex cellulosic materials from trees. Hence, endophytic fungi associated with orchids could be rich source for production of carboxymethyl cellulase (CMCase) enzyme. Cellulase has vast industrial applications in alcohol fermentation, food industries, paper pulp industries and textile industries (Van Dyk &

Pletschke 2012). The cellulase also has a major role in biomass utilization to meet global energy requirement.

The reports on production of extracellular enzymes from orchid endophytic fungi are meager hence, the present work was undertaken to screen and optimize culture conditions for maximum cellulase production from endophytic fungus *Colletotrichum gloeosporioides* associated with *Cymbidium aloifolium.*

MATERIAL AND METHODS

Isolation and screening of endophytic fungi for CMCase

The endophytic fungus associated with root, leaf and flower of *Cymbidium aloifolium* was isolated on potato dextrose agar (PDA) with 50µg/ml tetracycline. The isolated endophytic fungus was screened for production of CMCase using glucose yeast extract peptone agar (GYPA) media amended with 0.5% carboxymethyl cellulose (CMC). The fungus inoculated petri dishes were incubated at room temperature for one wk; the plates were flooded with 0.2% aqueous congo-red solution and destained with 1M NaCl for 15 min and appearance of yellow zone around the colony indicated the production of CMCase. The diameter of hydrolysis zone and fungal colony were measured; enzyme index was calculated using the following formula (Florencio et al. 2012):

Enzyme index = Diameter of hydrolysis zone / Diameter of fungal colony.

Optimization of parameters for production of CMCase

The different culture parameters like temperature, pH, carbon and nitrogen sources were optimized for maximum production of CMCase. 0.5 ml of 10^6 fungal spores/ml were inoculated into 100 ml of basal media, incubated for a wk under different physical and chemical parameters to determine optimum conditions for maximum CMCase production (El-Bondkly & El-Gendy 2012).

The optimum temperature for CMCase production was determined by incubating fungus at 20^0 C, 25^0 C, 30^0 C, 35^0 C and 40^0 C for a wk. The optimum pH for CMCase production was determined by varying pH of media from pH 4–8. The basal media amended with various carbon sources (2% w/v) such as rice bran, wheat bran, cassava flour, CMC and maltose; the optimum carbon source for production of CMCase was determined. The basal media was amended with different nitrogen sources (0.3% w/v) such as ammonium nitrate, peptone, yeast extract, tryptone, beef extract and optimum nitrogen source for production of CMCase was determined. The fungal biomass obtained after filtration was dried in hot air oven at 80^0 C for 16 h and mycelia dry weight was expressed as mg/ml.

CMCase assay

CMCase activity was determined by adding 1 ml of crude enzyme extract to 1 ml of 1% CMC in citrate buffer (50 mM), pH 5. The mixture was incubated at 60^0 C for 30 min and then 1 ml of

DNS was added, mixed and boiled in water bath for 10 min, 2.5 ml of distilled water was added and absorbance measured at 540 nm. The standard graph was plotted and the concentration of reducing sugar released by crude enzyme filtrate was calculated using equation obtained. The one unit (U) of cellulase activity is the amount of enzyme required to liberate one micromole of reducing sugar from substrate per min under assay conditions (Zhang et al. 2009).

Units/ml cellulase enzyme activity = µmole of reducing sugar liberated/Total incubation time in minutes * volume of crude enzyme extract in ml

Statistical analysis

The experiments were performed in triplicates, means of enzyme index were analyzed statistically and Duncan multiple range test (DMRT) was carried out using SPSS software version 20; IBM, Armonk, NY, USA (Barrett et al. 2012).

OBERVATIONS

80% of endophytic fungus isolated from different parts of *Cymbidium aloifolium* produced CMCase enzyme; highest enzyme index was 7.99 by *Colletotrichum gloeosporioides*, 4.5 by *Trichoderma* sp., 3.6 by *Fusarium oxysporum* and lowest enzyme index was 1.09 by *Curvularia lunata*.

Optimization of culture parameters for production of CMCase

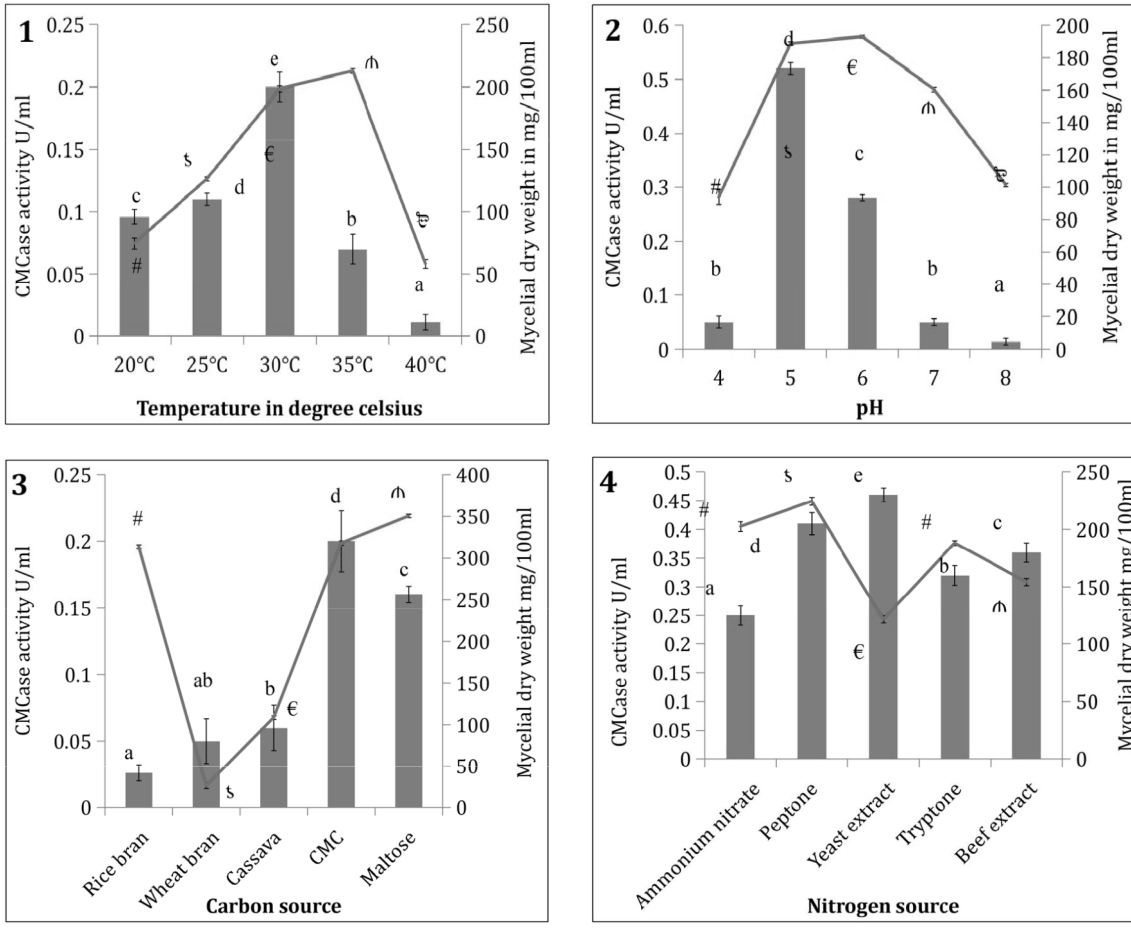
The highest CMCase production with enzyme index of 7.99 was recorded in *Colletotrichum gloeosporioides* hence, optimization of culture parameters for maximum production of CMCase was done with this fungus.

Effect of temperature on CMCase

The activity of CMCase enzyme by endophytic fungus, *Colletotrichum gloeosporioides* significantly varied with different temperatures. The highest enzyme activity of 0.2 ± 0.012 U/ml was at 30^0 C followed by 0.11 ± 0.005 U/ml at 25^0 C, 0.096 ± 0.006 U/ml at 20^0 C, 0.07 ± 0.012 U/ml at 35^0 C and 0.011 ± 0.006 U/ml at 40^0 C (Fig. 1). The highest fungal biomass (213 ± 2 mg/100 ml) was at 35^0 C followed by 198.33 mg/100 ml at 30^0 C, 126.33 ± 1.52 mg/100 ml at 25^0 C, 74.66 ± 4.5 mg/100 ml at 20^0 C and 58.33 ± 3.51 mg/100 ml at 40^0 C. There was no cellulase activity and fungal growth at temperature of 15^0 C and 45^0 C as this fungus is mesophilic and grows in the temperature range of 20–40^0 C.

Influence of pH on CMCase

In the present study, pH influenced the production of CMCase enzyme by *Colletotrichum gloeosporioides*. The optimum pH for cellulase production was 5 and exhibited the cellulase activity of 0.52 ± 0.011 U/ml followed by pH 6 with 0.28 ± 0.006 U/ml activity, pH 4 and pH 7 with activity of 0.05 ± 0.011 U/ml and 0.05 ± 0.006 U/ml; pH 8 exhibited least cellulase activity of 0.014 ± 0.006 U/ml (Fig. 2). The fungal biomass varied with the different pH conditions and it did not correlate with cellulase activity. The fungal growth was luxuriant at pH 6 with biomass



Figs 1– 4: Optimization of culture parameters for CMCase production in *Colletotrichum gloeosporioides.* 1. Optimization of temperature conditions for maximum CMCase production. 2. Optimization of pH conditions for maximum CMCase production. 3. Optimization of carbon sources for maximum CMCase production. 4. Optimization of nitrogen sources for maximum CMCase production.

The values represent mean of enzyme index ± SD, n = 3; Mean values followed by the same letter and symbol are not significantly different according to DMRT at $p < 0.05$.

of 192.8 ± 0.85 mg/100 ml followed by 188.53 ± 1.15 mg/100 ml at pH 5, and 160.4 ± 1.37 mg/100 ml at pH 7. The biomass produced was lesser at pH 4 and 8 suggesting that pH had a significant influence on CMCase activity and fungal biomass.

Influence of carbon sources on CMCase

Different carbon sources played an imperative role in production of cellulase by endophytic fungi. The cellulase activity and fungal biomass varied with different carbon sources. In the present study, CMC as the carbon source,

exhibited highest enzyme activity (0.2 ± 0.023 U/ml) followed by maltose (0.16 ± 0.006 U/ml), cassava flour (0.06 ± 0.017 U/ml), wheat bran (0.05 ± 0.017 U/ml) and rice bran (0.026 ± 0.006 U/ml) (Fig. 3). The fungal biomass varied with different carbon sources; maltose as carbon source produced highest fungal biomass (350.33 ± 2.08 mg/100 ml) followed by CMC (317.66 ± 2.51 mg/100 ml), rice bran (313.36 ± 2.09 mg/100 ml), wheat bran (246.06 ± 3.55 mg/100 ml) and cassava flour (108.46 ± 2.15 mg/100 ml).

Influence of nitrogen sources on CMCase

The yeast extract exhibited highest cellulase activity (0.46 ± 0.012 U/ml) followed by peptone (0.41 ± 0.019 U/ml), beef extract (0.36 ± 0.017 U/ml), tryptone (0.32 ± 0.017 U/ml) and ammonium nitrate (0.25 ± 0.017 U/ml) (Fig. 4). The peptone as nitrogen source produced highest fungal biomass (224.43 ± 3.37 mg/100 ml) followed by ammonium nitrate (202.4 ± 4.132 mg/100 ml), tryptone (188.33 ± 2.08 mg/100 ml), beef extract (154.33 ± 3.21 mg/100 ml) and yeast extract (121.46 ± 3.07 mg/100 ml).

DISCUSSION

The highest CMCase production was recorded in *Colletotrichum gloeosporioides* and optimization of culture parameters like temperature, pH, carbon and nitrogen sources for maximum production of CMCase was done with this fungus.

The present findings agree with those of Gao et al. (2010) who have reported that, hydrolytic enzymes such as cellulase produced by endophytes help to hydrolyze variety of polymeric compounds associated with plant cell walls. The structures of fungal cellulase are simpler when compared to bacterial cellulase. The fungal cellulase characteristically has two separate domains; a catalytic domain and a cellulose binding module joined by short polylinker to catalytic domain at N-terminal (Bayer et al. 1998). The microbial cellulase has potential applications in various industries like paper, pulp, textile, bioethanol, laundry, food processing, wine brewing, agriculture, detergent and waste management (Kuhad et al. 2011). The vast applications of cellulase in different industries have invoked interests of both academic and industrial research groups in improving production and economics of cellulase.

The present study is in congruent with those of Srilakshmi et al. (2017) who reported maximum production of cellulase in *Purpureocillium lilacinum* at 30^0 C. Mrudula & Murugammal (2011) also reported 30^0 C as the optimum temperature for production of cellulase by *Aspergillus niger* but, Pathania et al. (2015) reported the optimum temperature for cellulase production in *Aspergillus* sp. at 28^0 C. The fungal biomass production did not correlate with cellulase activity.

The enzymes have an optimum pH in which they exhibit maximum activity and their activity decreases with higher or lower pH values (Lehninger et al. 1993). The present findings concur with those of Sethi & Gupta (2014) who reported pH 5 as optimum for production of cellulase enzyme but, El-Hadi et al. (2014)

reported pH 7 as optimum for production of cellulase by *Aspergillus hortai*.

Pathania et al. (2015) reported that fructose was found to be the best carbon source followed by dextrose, cellulose, sucrose and galactose for cellulase production. The present investigation agrees with that of Harrer et al. (1983) who reported the effect of CMC on extracellular enzyme formation in *Trichoderma pseudo-koningii*.

The influence of different nitrogen sources such as yeast extract, peptone, beef extract, tryptone and ammonium nitrate on production of cellulase by *Colletotrichum gloeosporioides* was determined in the present study. The present findings agree with those of Prasanna et al. (2016) who reported yeast extract followed by peptone as the best nitrogen source for the production of cellulolytic enzymes by *Penicillium* sp. and Gupta et al. (1996) who reported enhanced cellulase production in the presence of peptone by *Volariella displasia.* Han et al. (2009) also reported peptone as the most promising and effective nitrogen source for cellulase production by *Penicillium waksmanii*. The fungal biomass content yielded by *Colletotrichum gloeosporioides* was higher on organic nitrogen than inorganic nitrogen sources.

From the results of the present investigation it becomes evident that endophytic fungi associated with epiphytic orchids are good reservoirs of economically important enzymes like cellulase and culture parameters have significant influence on the production of extracellular enzymes and optimization leading to enhanced production of enzymes.

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