Karyotype Analysis in 2 Morphological Forms of *Xanthium strumarium* L.

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Summary The 2 morphological forms of *Xanthium strumarium* L., namely “the purple form and the green form, available in Bangladesh were studied cytogenetically for critical analysis after staining with orcein, CMA and DAPI. Both of the forms were found to possess $2n=36$ chromosomes. The centromeric formula was determined $27m+9sm$ in the purple form and $23m+13sm$ in the green form. The purple form showed more heterogeneous karyotype than the green form. No satellite was found in these 2 forms after orcein staining. However, after CMA-staining a pair of conspicuous satellite was found in the purple form, which was absent in DAPI-staining. In this form (purple), 2 interstitial CMA-positive bands were found which showed deep DAPI-negative band. In the green form, 6 CMA-positive upper terminal bands were found. Out of 6 CMA-positive bands 2 showed deep and complete DAPI-negative bands whereas another 2 bands showed partial DAPI-negative bands. The reversible banding indicated the presence of fully GC-rich repeats of those portions. These chromosomes could be used as marker for respective forms. The fluorescent banding revealed the occurrence of inversion in pair II of the purple form. Thus the 2 forms have distinct and unique karyotypes. The comparative karyotype analysis would provide strong support for authentic taxonomic diversification of these 2 forms of *Xanthium strumarium*.

Key words Fluorescent banding, Karyotype, *Xanthium*, Asteraceae.

The genus *Xanthium* L. belongs to the family Asteraceae. The members of this genus are distributed globally, however are most frequently in to be found tropical and sub-tropical regions (Weaver and Lechowich 1982). They are wide-spread in America, Canada, Mexico, Malaysia, Indonesia and India. In Bangladesh, species under this genus is widely distributed throughout the country especially on the bank of rivers and paddy fields.

The taxonomic study of the members of this genus is difficult and confusing. For example, Caius (1986) reported that the genus *Xanthium* includes 25 species. However, according to Weaver and Lechowich (1982), there are 20 species belonging to this genus. Thus confusion about the species number of this genus still exists. Love and Dansureau (1959) revised the genus *Xanthium* and reduced the number of species to only 2, namely *X. strumarium* L. and *X. spinosum* L. They mentioned that due to phenotypic plasticity in a number of features, the members showed different phenotypes and creating confusion regarding their taxonomic rank. Prain (1903) described that the 2 species *i.e.* *X. strumarium* and *X. spinosum* were found in undivided Bengal. However, Oudhia and Dixit (1994) reported *X. indicum* and *X. strumarium* from India. The species *Xanthium strumarium* has many types with different characteristics that vary in response to environmental factors. These types are presently grouped into complexes within the species (Holm *et al.* 1991). Therefore, a confusion regarding the taxonomy about this genus is present.

In Bangladesh, only 1 species, namely *Xanthium strumarium*, has so far been reported (Hossain 2008). The species found in Bangladesh has 2 morphological forms. These are i) the pur-

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ple form (locally known as 'Lal ghagra'), ii) the green form (locally known as 'ghagra'). *Xanthium strumarium* has much ethno-botanical importance. Recently some toxic effects of *Xanthium strumarium* have been reported from Bangladesh. After consuming the seed and seedlings of *X. strumarium*, an outbreak including vomiting and unconsciousness occurred in the people of the north-eastern part of Bangladesh in 2007, including some fatalities (Gurley *et al.* 2010). Although both the forms were reported as poisonous at the seedling stage, the purple form is more toxic (Dr. Mohammed Zashim Uddin, personal communication).

Taxonomists are still facing problems for the authentic identification of the members of this species. It is well known that where morphological features are not adequate, cytological research may solve the taxonomic problem.

Among cytological studies, karyotype analysis is the best option, because the karyotype is stable and is a constant parameter specific to a particular organism. However, it is not easy to differentiate between similar karyotypes having the same $2n$ chromosome number and almost equal centromeric position by conventional analysis. In such a case, critical karyotype analysis should be applied.

Fluorescent chromosome banding is another strong cytological tool for differentiating between symmetric karyotypes. Two common fluorochromes *i.e.* CMA and DAPI are widely used. CMA binds to GC-rich repeats and gives a characteristic yellow color while DAPI binds to AT-rich repeats fluorescing a characteristic blue color. With the help of fluorescent banding even a single chromosome could be identified (Huq *et al.* 2007, Khandaker *et al.* 2007, Sultana *et al.* 2007, Akter and Alam 2005, Alam and Zerin 1998).

The above mentioned critical cytological parameters should apply for the authentic characterization of the 2 forms of *Xanthium strumarium* found in Bangladesh. Keeping this in mind, the present research was undertaken. The major aims of this research are:

- i) to compare the orcein-stained karyotypes of the 2 forms;
- ii) to compare the CMA and DAPI banding patterns of the 2 forms;
- iii) to find out marker chromosomes (if any) and;
- iv) to ascertain the taxonomic status of these 2 forms.

Materials and methods

Two morphological forms of *Xanthium strumarium*, *viz.* the purple form and the green form, were used in this study. Both the forms are an erect annual herb with short stout hairy stem. The plants of purple and green form were collected from the district of Gazipur and sea beach of Cox's Bazar, respectively. The purple form is bigger than the green form. At the seedling stage the leaves and petioles of this form are purple in color. At maturity the petioles and the veins of the leaves remain deep purple in color, in addition, the basal parts of the stem are deep purple. On the other hand the green form is smaller than the other form. The seedlings of this form have no purple color although at juvenile stage the basal part of the stem is comparatively less purple than the other form. At maturity, it becomes completely green. The morphological features of the 2 forms are very much stable. The plant materials were grown and maintained in the Botanic Garden, Department of Botany, University of Dhaka.

Healthy roots were collected and pretreated with 8 Hydroxy-quinoline for 1 h and 30 min at 18°C followed by 15 min fixation in 45% acetic acid at 4°C. These were then hydrolyzed in a mixture of 1 N HCl and 45% acetic acid (2:1) at 60°C for 7 s. The root tips were stained and squashed in 2% aceto-orcein. For fluorescent banding, Alam and Kondo's (1995) method was followed with slight modification. After hydrolyzing and dissecting, the materials were squashed with 45% acetic acid. The cover glasses were removed quickly on dry ice and allowed to air dry for at least 48 h before study. The air-dried slides were first pre-incubated in McIlvaine's buffer (pH 7.0) for 30 min

followed by Distamycin A (0.1 mg/ml) treatment for 10 min in a humid chamber. The slides were rinsed mildly in McIlvaine's buffer supplemented with MgSO_4 (5 mM) for 15 min. One drop of CMA (0.1 mg/ml) was added to the materials for 15 min in a humid chamber and rinsed with McIlvaine's buffer with Mg^{2+} for 10 min. Slides were mounted in 50% glycerol and kept at 4°C for overnight before observation. These were observed under Nikon (UFX-IIA) fluorescent microscope with Blue Violet (BV) filter cassette. For DAPI-staining, after 48 h of air drying, the slide was first pre-incubated in McIlvaine's buffer (pH 7.0) for 20 min in a humid chamber. The slides were treated in actinomycin D (0.25 mg/ml) for 10 min. The slides were immersed in DAPI solution (0.01 mg/ml) for 20 min and mounted with 50% glycerol. These were observed under Nikon (UFX-IIA) fluorescent microscope with Ultra Violet (UV) filter cassette.

Results and discussion

Conventional Karyotype

Both the forms of *Xanthium strumarium* were found to possess $2n=36$ chromosomes (Figs. 1a, b). $2n=36$ chromosomes of this species was reported earlier (Ishikawa 1916, Mehra *et al.* 1965). Therefore, the present count confirmed the earlier reports on $2n$ chromosomes number. The 2 forms have different centromeric formulae such as $27m+9sm$ for purple form and $23m+13sm$ for green form (Table 1). The range of chromosomal length and total $2n$ length showed that the purple form possessed bigger chromosomes. The centromeric formulae, range of chromosomal length and total length of $2n$ complements clearly indicated distinct karyotypes of these 2 forms of *Xanthium strumarium*.

CMA-karyotypes

In the purple form, 2 interstitial CMA-bands were observed 1 in each member of pair II (Figs. 1c, g). The 2 interstitial CMA-bands were not at the similar sites, 1 in the long and another in the short arm of the respective chromosomes. This result indicated the probable occurrence of an inversion. In addition, 2 CMA stained satellites were observed in pair VIII (Figs. 1c, g). In contrast, 6 big and bright terminal bands were observed in the green form (Figs. 1d, i). The bands of this form were much bigger than those of the purple form and occupied about the length of short arms of the respective chromosomes. Schweizer (1976) stated that CMA-binds with GC-rich repeat. The present finding revealed that the short arms of these chromosomes were almost composed of GC-rich repeats. The total amount of GC-rich repeats was about 4 times more in the green form (Table 1). Therefore, these 2 forms of *Xanthium strumarium* have distinct CMA-karyotypes.

Reversible fluorescent banding

When a region of chromosome fluoresce with a fluorochrome (positive banding) and does not take another counter fluorochrome (negative banding), this kind of banding feature is known as reversible banding. Reversible fluorescent banding pattern was observed in pair II of the purple form while in pair III and VIII of the green form. In the purple form, the 2 interstitial CMA-positive bands showed deep DAPI-negative bands at the same location (Figs. 1c, e, g, h, arrow). As a result, the DAPI-negative region looked like a gap at the interstitial regions of the respective chromosomes. In the green form, the 2 terminal CMA-positive bands in pair III and VIII showed DAPI-negative bands at those regions. As a consequence, the DAPI-negative chromosomes looked much smaller (something missing from the terminal end of short arm) when compared to the CMA-positive chromosomes (Figs. 1d, f, i, j, arrow). Reversible fluorescent banding (CMA-positive and DAPI-negative) indicated the presence of complete GC-rich repeats in those regions of chromosomes (Schweizer 1976, Alam and Kondo 1995, Alam *et al.* 1996). The reversible banding was so unique that these chromosomes could easily be used as the marker chromosomes for the respective karyotypes.

Fig. 1. Mitotic chromosomes of 2 forms of *Xanthium strumarium* L. stained with orcein, CMA and DAPI. a. Orcein-stained mitotic metaphase chromosomes of the purple form. b. Orcein-stained mitotic metaphase chromosomes of the green form. c. CMA-stained mitotic metaphase chromosomes of the purple form. d. CMA-stained mitotic metaphase chromosomes of the green form. e. DAPI-stained mitotic metaphase chromosomes of the purple form. f. DAPI-stained mitotic metaphase chromosomes of the green form. g. CMA-stained karyotype prepared from mitotic metaphase chromosomes of the purple form. h. DAPI-stained karyotype prepared from mitotic metaphase chromosomes of the purple form. i. CMA-stained karyotype prepared from mitotic metaphase chromosomes of the green form. j. DAPI-stained karyotype prepared from mitotic metaphase chromosomes of the green form. Bar=10 μ m.

Satellite

In the purple form, a pair of satellites was observed on the short arms 1 in each member of pair VIII with CMA-stain (Figs. 1c, g). However, no satellites were found in orcein and DAPI-stains (Figs. 1a, h). The presence of satellites in CMA (CMA-positive) and the absence of such in DAPI (DAPI-negative) indicates that the satellite regions were completely composed of GC-rich repeats.

Table 1. Comparative orcein, CMA and DAPI-stained karyotype analysis in 2 forms of *Xanthium strumarium* L.

Forms	2n	Total length of 2n chromosome complement (μ m)	Karyotype formulae	Range of chromosomal length (μ m)	No. of CMA-positive bands	No of CMA-positive satellites	No. of DAPI-negative bands	% of GC-rich repeats
Purple form	36	397.7	27m+9sm	8.00–14.32	2	2	4	1.31
Green form	36	304.9	23m+13sm	6.53–11.06	6	0	4	4.52

GC-rich satellites were reported earlier in different species (Alam and Kondo 1995, Sultana and Alam 2007). Why satellites were not found in orcein staining is now a question. About 50 well-spread mitotic metaphase plates were observed to locate the satellite after orcein staining. No satellite was found in any case. Therefore, absence of satellite in the orcein staining mitotic metaphase was not a technical error. It may be concluded that the satellite regions were composed of stain-specific DNA segments. Alam and Kondo (1995) reported stain-specific chromosomal segments in *Drosera* spp. Moreover, stain-specific satellite was observed by Sultana and Alam (2007). The earlier and the present finding suggested the presence of stain-specific satellite region in same plant species.

Karyotype characterization

The foregoing discussion clearly indicated that despite of same 2n chromosomes number, the 2 forms of *Xanthium strumarium* possessed distinct karyotypic features specific to each form. Therefore, the 2 forms of *Xanthium strumarium* could be characterized by comparative karyotype analysis. The present data of comparative karyotype analysis would also be used for authentic taxonomic diversification of these 2 forms of *Xanthium strumarium*.

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