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CARITOTIPIC CHARACTERIZATION OF *ECHINACEA ANGUSTIFOLIA* D.C. PLANTS MAINTAINED IN THE GENEBANK FROM S.C.D.L. BACAU

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INTRODUCTION

All species of *Echinacea* are herbaceous, perennial flowering plants of the composite family (Asteraceae). Generally, a basal rosette of petiolate leaves and one to several annual stems arise from an underground, perennial rootstock. A single taproot is characteristic of all species except for *E. purpurea*, which has a fibrous root system. The stems terminate in a long-lasting and mildly fragrant inflorescence. The disk flowers are attached to a conical, hemispherical or occasionally flattened receptacle. The disk itself may also be flattened, conical, or hemispheric. Surrounding the capitulum is an involucre of 3 to 4 series of imbricating bracts (phyllaries). Each disk floret is subtended abaxially by a modified bract, the palea, which protrudes beyond the 5-lobed corolla.

The disk florets are protandrous and development and anthesis follow the typical centripetal pattern of development of the composite family.

The whorls of sharply tipped palea give a distinctive look to the capitulum; indeed, the genus name is derived from the Greek word *echinos*, for hedgehog. Pubescence on the stems, leaves and bracts ranges from hispid, hirsute and strigose to glabrous and varies among the species. The sterile ray flowers have strap-shaped ligules with colors that range from white, pink, magenta and purple to yellow.

The majority of taxa are diploid (n = 11). *E. pallida* and some populations of *E. angustifolia* var. *strigosa* McGregor are tetraploid (n = 22) (McGregor 1968). A complete understanding of the reproductive biology and breeding system is lacking; the presumed sporophytic self-incompatibility system is not perfected in this genus. Every species of *Echinacea* self pollinates to some degree, *E. purpurea* more so than the others (McGregor pers. commun. 1997). A mixed breeding system has been recently demonstrated for *E. angustifolia* var. *angustifolia* (Leuszler et al. 1996).

The size of chromosomes and their morphology are important indicators used to identify the evolutionary relationships between different plant species (Albrecht, 2000). The field of applicability of genetics studies is extremely broad, starting from fields of fundamental study (taxonomy), reaching up to applicative fields (eg plant breeding). Genetic characterization is important for species identification or hybrid population analysis. Genetic determinations include chromosomal counting and karyotype determination, mitotic indexing, and mitotic anomaly analysis.

These studies allow to determine the following parameters (McKeown, 2004): the number, shape and size of the chromosomes in mitosis and accomplisment of the karyotype of a specific species; study of the physical or chemical mutagens influence on karyotype; determining the degree of ploidy of plants that either have been treated with different poliploidizing substances or have undergone changes in their cultivation under "in vitro" conditions; determining chromosome homology by studying metaphase 1 of meiosis as well as dissociating chromosomes in other phases in plants with varying degrees of ploidy and interspecific hybrids; the study of aneuploid organisms and the way genes are placed on chromosomes; intra and inter-specific transfer of genes and chromosomes, or chromosomal segments, etc.

MATERIAL AND METHODS

The biological material used in the present cytogenetic study is represented by the radicular meristems of the plantlets resulting from the germination of the seeds of *Echinacea angustifolia* D.C.

In order to ensure the accuracy of the data, seeds from three different plants were used, the results obtained by the species representing the mean of the values obtained at each of the three plants, which are still considered as working variants.

Thus, *Echinacea angustifolia* seeds were placed in petri dishes in germinators at a temperature of 25^0C. After germination, roots with dimensions between 1 and 5 mm were harvested from the resulting plants.

Fig. 1. Seeds of *Echinacea angustifolia* D.C. utilised as biological material

The preparation of biological material for cytogenetic studies was carried out according to the data presented by the literature. Thus, two working methods were used to determine the best coloring method, namely the Schiff Reagent staining method and Carr's Reagent staining method.

After hydrolysis, on remove HCl from the vials and add 2-3 ml of Schiff or Carr reagent. As a result of the chemical reaction that occurs between the aldehyde groups of the DNA released by hydrolysis and the basic fucsine, after 15-30 minutes, the meristematic region at the top of the roots is colored red-violet. To enhance coloration, the roots can be left in the Schiff reagent for 1-2 hours.

Placing the material on the blade is done as follows: a drop of acetic water is placed at the middle of the blade. Near the drop, with a spattered needle, the colored meristematic tip of a root is cut and dropped. Over the drop of acetic water containing the root tip is placed a glycerinated albumen glycerinate and quickly passed through the flame of a gas or alcohol lamp for coagulation of albumin (the use of glycerol albumin is not absolutely necessary, its role being merely to prevent dispersing chromosomes from the cells that break through the blade display).

Chromosome examination and photographing were performed on the Hund microscope at a magnitude of 1000x.

For karyotype analysis, after colchicine prefixing, at least 7 high-quality cell metaphases (in which the chromosomes were very well-sampled) were analyzed for chromosome length in mitosis for each seed source. Measurement of chromosomes was done by correlating the measurements made using the microscope micrometer and the measurements made in Adobe Fotoshop 5.

RESULTS AND DISCUSSIONS

Seeds of *Echinacea angustifolia* D.C. have germinated under the conditions we tested masively. Thus, 9 days after their placement in petri dishes, on wetted filter paper, the seeds germinated and could be used for root harvesting and initiation of cytogenetic studies. The obtained results are presented in table no. 1 and fig.1.

Table 1. The results of the tests for seeds germination of *Echinacea angustifolia* D.C.

Variant	Working variant	No. of inoculated seeds	No. of germinated seeds	%
Var. 1	*Echinacea angustifolia* D.C. – planta 1	50	48	96%
Var. 2.	*Echinacea angustifolia* D.C. – planta 2	50	49	98%
Var. 3.	*Echinacea angustifolia* D.C. – planta 3	50	50	100%

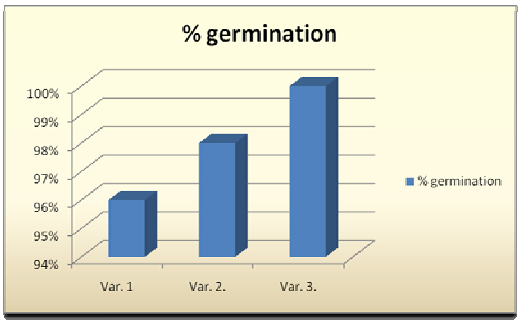

Fig. 1. Graphycal representation of germination dynamics

After germination – fig. 2, some of the root seed meristems were used to establish the karyotype. For this purpose, part of the seeds were subjected to the prefixing process (in 0.02% colchicine aqueous solution for 4 hours), necessary for the hypercontraction and dispersion of the chromosomes in the metaphase, and the rest of the seeds were passed directly into the fixator.

Fig. 2. Seeds of Echinacea angustifolia D.C. germinated on filter paper, used in cytogenetic studies

It is well known that *Echinacea angustifolia* is a diploid species (2n = 22). The number and morphology of the *Echinacea angustifolia* chromosomes identified in the radicular meristems of the elite plants selected in the breeding works from S.C.D.L. Bacau falls into the results presented by the literature (Kim, 2004), with no significant changes in their number, size or morphology – table 2.

Table 2. Results of chromosome measurements performed in Echinacea angustifolia D.C.

Pair of chromosomes	Total lenght*	Long arm length*	Short arm length*	Centromeric index **	Arm ratio***	The type of chromosome ****
I	5.86	3.37	2.49	42.49	1.35	m
II	5.69	3.20	2.49	43.76	1.28	m
III	4.94	3.90	1.04	21.05	3.75	st
IV	4.93	2.73	2.20	44.62	1.24	m
V	4.95	3.67	1.28	25.85	2.86	sm
VI	4.87	3.48	1.39	28.54	2.50	sm
VII	4.86	2.57	2.29	47.11	1.12	m
VIII	4.55	3.85	0.70	15.38	5.5	st
IX	4.34	3.36	0.98	22.58	3.42	st
X	4.39	3.99	0.31	7.06	12.87	t
XI	4.13	3.40	0.73	17.67	4.65	st

* The chromosome type nomenclature is based on data provided by the literature, and the chromosome length is expressed in µm.
** Centromeric index = ratio of short arm length to total chromosome length, expressed as a percentage
*** Arm ratio = long arm length: short arm length
*** m = with centrometer in the median area; sm = with the centrometer in the submedia zone; st = with the centromer in the subterminal region; and t = with the centrometer in the terminal area.

Generally, the chromosome sizes varied between 4.12 and 5.83 µm. Four pairs of chromosomes with centromers in the median area and two pairs with centromers in the sub-median area were identified. Of the remaining five pairs of remaining chromosomes, four have centromers in the sub-terminal area, and the tenth pair has the centromer in the terminal region. Figure 3 shows a typical mitotic metaphase.

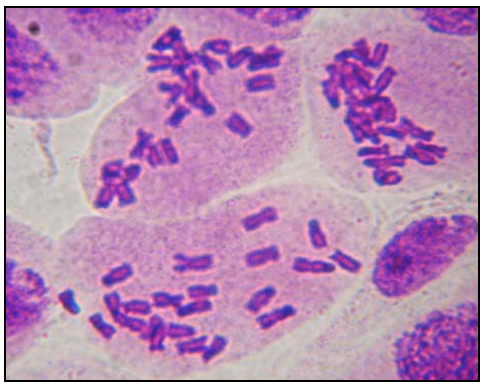

Fig. 3. Metaphases chromosomes of *Echinacea angustifolia* D.C. identified in root systems of selected elite plants

No secondary constrictions giving rise to satellites were observed.

CONCLUSIONS

Echinacea angustifolia is a diploid species (2n = 22). The number and morphology of the *Echinacea angustifolia* chromosomes identified in the radicular meristems of the elite plants selected in the breeding works from S.C.D.L. Bacau falls into the results presented by the literature (Kim, 2004), with no significant changes in their number, size or morphology.

The results obtained showed that the chromosome sizes varied between 4.12 and 5.83 µm. Four pairs of chromosomes with centromers in the median area and two pairs with centromers in the sub-median area were identified. Of the remaining five pairs of chromosomes, four have centromers in the sub-terminal area, and the tenth pair has the centromer in the terminal region.

ABSTRACT

The research presented in this paper focused on the cariotypic characterization of *Echinacea angustifolia* D.C. existing in the collection from S.C.D.L. Bacau. Genetic characterization is important for species identification and hybrid population analysis. Genetic determinations include chromosomal counting and karyotype determination, mitotic indexing, and mitotic anomaly analysis.

The biological material used in the present cytogenetic study is represented by the radicular meristems of the plantlets resulting from the germination of the seeds of *Echinacea angustifolia* D.C.

The preparation of biological material for cytogenetic studies was carried out according to the data presented by the literature. Thus, two working methods were used to determine the best coloring method, namely the Schiff Reagent staining method and Carr's Reagent staining method. Measurement of chromosomes was made by correlating the measurements made using the microscope micrometer and the measurements made in Adobe Fotoshop 5.

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