Karyomorphological Studies on Halophytic Plants

I. Some taxa of Chenopodium

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One of the problems of chromosome morphology still to be solved is whether the chromosomes of plants which occur in such peculiar places as salt marsh show specialized morphology or not. Several papers have been published on this problem concerning polyploidy (Hagerup 1932, Shimotomai 1933, Tischler 1934, etc.) and chromosome size (Heitz 1926).

Halophytic plants grow within the influence of salt water. The influence of salt water is evidently so severe that the plants from common soils suffer damage when they are put in the same conditions as halophytic plants. There are some papers on the chromosomes of halophytic plants (Wulff 1937, Hara 1952, Uotila 1973, Keener 1974). According to these papers chromosome numbers are reported, but no mention is made on peculiar characteristics of chromosomes.

For the study of chromosomal characteristics of halophytic plants it is necessary to compare phylogenetic allies of the respective species. In the present investigation the halophytic plants are dealt with its phylogenetic allies. In this report five taxa of Chenopodium in Japan are investigated.

Material and method

All materials used were collected in natural growing places of the respective taxa. The places are shown in Table 1. Most of the clones were grown in the experimental garden of Hiroshima University, and some of them in Ehime University. Validating specimens of the clones studied karyomorphologically were deposited in the Herbarium of the Botanical Institute of Hiroshima University.

Observations of chromosomes were made in root tips and flower buds from clones grown in pots or garden. Observations on the chromosomes at resting stage and mitotic stage were made in the periblem cells. The root tips were fixed in 45% acetic acid for 10 min. at 10°C, hydrolysed in a mixture of 45% acetic acid and 1 N-HCl (1:2) for 15 sec. at 60°C, and stained with 1% aceto-orcein. In order to observe the position of the centromeres, the chromosomes were shortened to about two-thirds in length through pretreatment with 0.002 M 8-hydroxyquinoline. Flower buds were fixed in acetic alcohol (1:3) for over 1 hr. at 10°C. Meiotic chromosomes were observed in PMCs. The chromosomes were stained with 1% aceto-orcein, applying the squash method.
Table 1. Localities, habitats and chromosome numbers of five taxa of *Chenopodium*

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Locality</th>
<th>Habitat</th>
<th>Chromosome number</th>
<th>Number of clones studied</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chenopodium glaucum</em></td>
<td>Riv. Nuta-gawa, Mihara City</td>
<td>salt marsh</td>
<td>9 18</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Hirao, Yamaguchi Pref.</td>
<td>salt marsh in salt-field disused</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td><em>C. acuminatum</em> var. <em>japonicum</em></td>
<td>Miyatojima, Miyagi Pref.</td>
<td>sandy path adjacent to salt marsh</td>
<td>36</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Amino, Kyoto Pref.</td>
<td>sandy gravel faced to Japan See</td>
<td>36</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Kise, Mie Pref.</td>
<td>sandy path faced to Pacific Ocean</td>
<td>36</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Shirasaki, Wakayama Pref.</td>
<td>sandy gravel faced to Pacific Ocean</td>
<td>36</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Kada, Wakayama City Takihama, Ehime Pref.</td>
<td>sandy gravel in salt-marsh disused</td>
<td>18 36</td>
<td>1</td>
</tr>
<tr>
<td><em>C. album</em></td>
<td>Miyatojima, Miyagi Pref.</td>
<td>sandy field</td>
<td>54</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Kumihama, Kyoto Pref.</td>
<td>soil bank faced to salt-field disused</td>
<td>54</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Matsunaga, Fukuyama City</td>
<td>soil bank</td>
<td>54</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Koi, Hiroshima City</td>
<td>soil bank adjacent to salt marsh</td>
<td>54</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Kuba, Otake City Kitadoi, Matsuyama City</td>
<td>clayey field</td>
<td>27 54</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Riv. Sigenobugawa, Matsuyama City</td>
<td>soil bank</td>
<td>54</td>
<td>1</td>
</tr>
<tr>
<td><em>C. album</em> var. <em>centro-rubrum</em></td>
<td>Ushita, Hiroshima City</td>
<td>road-shoulder</td>
<td>54</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Kitadoi, Matsuyama City</td>
<td>clayey field</td>
<td>27 54</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Riv. Shigenobugawa, Matsuyama City</td>
<td>soil bank</td>
<td>54</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 1. (cont.)

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Locality</th>
<th>Habitat</th>
<th>Chromosome number</th>
<th>Number of clones studied</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. ambrosioides</em></td>
<td>Riv. Mori, Iyo City</td>
<td>sandy sea shore</td>
<td>54</td>
<td>1</td>
</tr>
<tr>
<td>var. <em>pubescens</em></td>
<td>Sendai City</td>
<td>road-shoulder near sea</td>
<td>32</td>
<td>1</td>
</tr>
<tr>
<td>Koi, Hiroshima City</td>
<td></td>
<td>sandy bank faced to salt marsh</td>
<td>32</td>
<td>5</td>
</tr>
</tbody>
</table>

Fig. 1. Photomicrographs of chromosomes in root tip cells (A–D) and PMC (E) of *Chenopodium glaucum*. A, resting stage. B, prophase. C, metaphase. D, metaphase treated with 8-hydroxyquinoline, $2n=18$. E, diakinesis, 9 bivalents. ×2800.
Observations

1. *Chenopodium glaucum* Linn., 2n=18

Four clones of this species were collected from sandy salt marsh in two localities (Table 1). They showed normal external morphology of leaves and flowers, except the stem length which varied from 5 cm to 30 cm. The four clones were found to be the same chromosome number, 2n=18 (Fig. 1), which verified the previous reports (Wulff 1937, Hara 1952 and so on).

Chromosomes at interphase formed many chromomeric granules and several chromocentral small blocks varied in number from 10 to 20 (Figs. 1A and 6A), thus showing chromocenter type nucleus (cf. Tanaka 1971a).

Chromosomes at prophase formed two distinct segments of heterochromatic and euchromatic segments (Fig. 1B). The heterochromatic segments located in proximal regions of both arms and transformed gradually into euchromatic segments located in distal ends showing chromomeric gradient. During all stages of prophase the heterochromatic segments were observed to be shorter than the euchromatic segments. At metaphase the euchromatic segments had split longitudinally, while the heterochromatic segments did not separate clearly (Fig. 1C). Chromosomes treated with 8-hydroxyquinoline showed a typical c-mitotic shape and the heterochromatic segments split longitudinally as well as the euchromatic segments (Fig. 1D).

Chromosomes at metaphase varied in length ranging from approximately 3 μm to 2 μm. The longest chromosome of the complement had the centromere situated in median position. The shortest chromosome had the centromere in submedian position. Two pairs of medium chromosomes had the centromere situated in subterminal position. Remaining five pairs had the centromere situated in median or submedian position. Satellites were found in a pair of the medium chromosomes which had the centromere in subterminal position. At prophase the satellite chromosomes were attached to a nucleolus by satellite and the distal region of short arms.

At meiosis nine bivalents were observed in all of the PMCs investigated (Fig. 1E). At diakinesis two of the bivalents were attached to a nucleolus. Most of the bivalents were ring shape, while few of them were rod shape. No secondary association was observed in the chromosomes of metaphase I.

2. *C. acuminatum* Willd. var. *japonicum* Franch. et Savat., 2n=36

Seven clones of this species were collected from six localities (Table 1). In all of the localities the clones were found growing in sandy path or sandy gravel along salt marsh or salt farm. The places where the clones occurred were observed to be above the zone of high tide, thus showing semi-halophytic habit.

Chromosomes in interphase nuclei showed many small chromocentral blocks varying in size (Figs. 2A and 6B). In most cells the blocks were counted to be 15–25. The chromatin other than the chromocentral blocks were either chromomeric or fibrous. The interphase nuclei were found to be of chromocenter type.
At prophase the chromosomes formed large heterochromatic segments in the proximal regions of both arms (Fig. 2B). The heterochromatic segments were further divided into two or more small heterochromatic segments. Distal ends of chromosomes were euchromatic. The heterochromatic segments transformed gradually into euchromatic segments. At mid-prophase the heterochromatic segments were observed to be longer than the euchromatic segments.

Fig. 2. Photomicrographs of chromosomes in root tip cells (A–D) and PMC (E) of Chenopodium acuminatum var. japonicum. A, resting stage. B, prophase. C, metaphase. D, metaphase treated with 8-hydroxyquinoline, 2n=36. E, diakinesis, 18 bivalents. ×2200.

2n=36 chromosomes were counted in all of the clones collected (Figs. 2C and D). The chromosomes varied in length from approximately 4 μm to 1.5 μm. The longest chromosome of the complement had the centromere situated in median position. The shortest chromosome of the complement had the centromere situated in submedian position. The 36 chromosomes were classified into 18 pairs which were grouped into eight pairs with median centromere, eight pairs with submedian centromere and two pairs with subterminal centromere. Satellites were found on the short arms of the two pairs with subterminal centromere. The satellite chromosomes were the medium chromosomes of the complement.
At prophase the satellite chromosomes were found attached to a nucleolus through the satellites and the short arms.

At meiosis 18 bivalents were observed in all of the PMCs studied (Fig. 2E). Most of the bivalents were ring shape, while few of them were rod shape. Four bivalents were found attached to a nucleolus. No secondary association was observed in all of the metaphase PMCs investigated.

Karyomorphological features of the interphase and the mitotic chromosomes of the present species were found to be similar to those of *C. glaucum*, except the chromosome number of the former was twice that of the latter. Therefore, the present species is considered to be a tetraploid with a basic number \( x = 9 \).

3. *C. album* Linn., \( 2n=54 \)

Nine clones of this species were collected from eight localities (Table 1). The clones were found growing in the common soils of sandy or clayey roadside and paths of farms.

Chromosomes in interphase nuclei were found to form the chromocenter type nucleus which was similar to those of the previous two species, while the size of the chromocenters varied more widely (Fig. 3A).

Chromosomes at prophase showed heterochromatic and euchromatic segments. All of the chromosomes of the complement had heterochromatic segments in proximal region and euchromatic segments in distal region. The heterochromatic segments showed a gradual transformation into the euchromatic segments.

Somatic chromosomes were found to be \( 2n=54 \) which confirmed the reports of Kjellmark (1934), Löve and Löve (1961) and so on (Fig. 3B). The chromosomes were smaller than those of the previous two species and varied in

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![Fig. 3. Photomicrographs of chromosomes in root tip cells (A, B) and PMC (C) of *Chenopodium album*. A, resting stage. B, metaphase, \( 2n=54 \). C, metaphase I, 27 bivalents. \( \times 2200 \).](image-url)
Fig. 4. Photomicrographs of chromosomes in root tip cells of *Chenopodium album* var. *centro-rubrum*. A, resting stage. B, prophase. C, metaphase. D, metaphase treated with 8-hydroxyquinoline, $2n=54$. $\times 2800$. 
length from about 2.5 µm to 1.3 µm at metaphase. The chromosomes had median or submedian centromere. A pair of the longest chromosome had median centromere. Satellites were found in two pairs of chromosomes which varied in length and had centromere subterminally.

At meiosis 27 bivalents were formed confirming the report of Uotila (1972) (Fig. 3C). Two of the bivalents were found attached to a nucleolus. No secondary association was observed in all of the PMCs investigated at metaphase I.

4. *C. album* Linn. var. *centrorubrum* Makino, 2n=54

Five clones of this species were collected from four localities (Table 1). They were found in sandy road-shoulder near rice fields. The external morphology of leaves and stems was similar to *C. album*, with the exception of pale violet young leaves.

Chromosomes in interphase nuclei showed many chromocenters which were similar in shape to those of previous *C. album* (Figs. 4A and 6C). Chromatin other than chromocenters were either chromomeric or fibrous. Thus, the chromosomes at interphase were found to be the chromocenter type.

Chromosomes at prophase showed long heterochromatic segments in proximal regions of both arms (Fig. 4B). Longer chromosomes of the complement had longer heterochromatic segments, while shorter chromosomes had shorter ones. The heterochromatic segments of longer chromosomes were found to be composed of several large chromomeric segments, and those of shorter chromosomes few chromomeric segments. Distal ends of chromosomes were euchromatic. The heterochromatic segments transformed gradually into euchromatic segments showing gradient of chromomeres. At mid-prophase the euchromatic segments of longer chromosomes were observed to be shorter (approximately 2/3) than the heterochromatic segments, while those of shorter chromosomes were approximately 1.5 times longer than the heterochromatic segments.

Chromosomes were counted to be hexaploid with 2n=54 in the root tip cells (Fig. 4D). The same chromosome number as *C. album* was found. The 2n=54 chromosomes at metaphase varied in length from 3.5 µm to 1.5 µm (Fig. 4C). Long chromosomes possessed the centromere situated in median or submedian position, while most of the shorter chromosomes possessed the centromere situated in subterminal position (Fig. 4D). The longest chromosome of the complement had the centromere situated in median position. Eight pairs of the shorter chromosomes had the centromere situated in subterminal position. The shortest chromosome of the complement was one of the chromosomes with a subterminal centromere.

Satellites were found in two pairs of the shorter chromosomes with subterminal centromere. The satellites and both arms of the satellite chromosomes were found to be heterochromatic at prophase. The satellite chromosomes were observed attached to a nucleolus by satellites and short arms.

The organizational pattern of the chromosomes was found to be similar to those of *C. album* and also to those of diploid and tetraploid species described in foregoing paragraphs. Therefore, *C. album* is presumably an autohexaploid with
a basic number $x=9$. However, if this species was a true autohexaploid, it should have six satellite chromosomes because each of the chromosome complement $x=9$ had a satellite chromosome. It is presumed that the chromosome complement of this hexaploid species lost its satellites leaving only a pair of satellite chromosomes. Thus, this species can be considered to be a partial allohexaploid.

Fig. 5. Photomicrographs of chromosomes in root tip cells (A–D) and PMC (E) of Chenopodium ambrosioides var. pubescens. A, resting stage. B, prophase. C, metaphase. D, metaphase treated with 8-hydroxyquinoline, $2n=32$. E, metaphase I, 16 bivalents. ×2800.
5. *C. ambrosioides* Linn. var. *pubescens* Makino, 2n=32

Six clones were collected from two localities (Table 1). The clones were found growing in sandy road-shoulder near sea.

Chromosomes at interphase showed round chromocenters which were weaker in staining than those of the previous species (Figs. 5A and 6D). They also formed many small chromomeres and fibrous chromatin which were similar to those of the previous species. Interphase chromosomes of this species were found to be of the chromocenter type.

Chromosomes at prophase had heterochromatic segments in proximal region (Fig. 5B). Distal ends of the chromosomes were euchromatic. Gradual gradient was observed between heterochromatic segments and euchromatic segments.

Chromosomes at prometaphase were observed to be strongly separated longitudinally into sister chromatids, thus forming X or Y shaped chromosomes attached at the proximal heterochromatic segments.

Somatic chromosomes were counted to be 2n=32 which confirmed that of Lorz (1937), Suzuka (1950) and so on (Fig. 5D). The 2n=32 was found to show a different series of basic number from the previous x=9. They varied in length from about 1.5 µm to 0.8 µm, smaller than those of previous four taxa (Fig. 5C). All of the chromosomes had median or submedian centromere (Fig. 5D). No chromosome with subterminal or terminal centromere was observed. Satellites were found in a pair of the smaller chromosomes with submedian centromere.

In comparison with the previous four taxa this species was found to differ in several features of metaphase chromosomes as follows: Different series of chromosome number, more smaller chromosomes, no terminal centromere, and earlier separation of sister chromatids. It is noticeable that in contrast to the difference in metaphase karyotypes the present *C. ambrosioides* showed the same chromocenter type nucleus as other four taxa.

Meiosis was normal. 16 bivalents were observed at diakinesis and metaphase I confirming the report of Hsu (1968) (Fig. 5E). At diakinesis proximal regions of the chromosomes of bivalents were heterochromatic and distal regions euchromatic. Chiasma was observed only in the distal euchromatic regions. Two bivalents were observed attached to a nucleolus. One of the bivalents was larger than the other and was found to have a satellite. In a few PMCs only larger bivalents were found attached to a nucleolus. At metaphase I, bivalents were either ring or rod form. Most bivalents were ring form. No secondary association was observed.

Discussion

The same basic number x=9 was found in four taxa showing an euploid series as follows: The halophytic *Chenopodium glaucum* was a diploid with 2n=18. The semi-halophytic *C. acuminatum* var. *japonicum* was a tetraploid with 2n=36. The non-halophytic *C. album* and *C. album* var. *centrorubrum*, on the other hand, were a hexaploid with 2n=54. Presumably the euploidy has played a role of ecological differentiation in the four taxa.
In contrast to the four taxa described above, C. ambrosioides var. pubescens had $2n=32$ indicating that this species belongs to a group with another basic number.

In comparison with the chromosome number, the morphological features of the chromosomes were found to be similar in the five taxa. In all of them the features of the condensed patterns of chromosomes in the resting stage (metabolic stage) were found to be grouped into the same type—the simple chromocenter type (Tanaka 1971a)—which was characterized by the few small chromocenters and many chromomeric granules (Fig. 6). In the mitotic stage the prophase chromosomes of the five taxa possessed similar early condensing segments in proximal region. The early condensing region showed gradual transformation into the late condensing region located in the distal region. Metaphase chromosomes of these species were found to be similar in size showing the homogeneous variation of size and symmetric arm-ratio. From the observations of the five taxa in Chenopodium it is concluded that there is no particular morphological features in the chromosomes of halophytic species.

Heitz (1926) made a proposal that the plants inhabited in cold-northern districts possessed large chromosomes. Opposed to this, Stebbins (1950) discussed that the large chromosomal characters showed some relation to the slower growth cycle of perennial herbaceous plants and that the large chromosomal plants reported by Heitz (1926) are regarded as the herbs with slower growth habit.

Delauney (1922) and Lewitzkey (1931) made a proposal that the morphological features of mitotic metaphase chromosomes have a close connection with the systematic phylogeny and evolitional trends. Many papers supporting this proposal have appeared. Tanaka (1971a, 1971b) made another proposal that in Orchidaceae the morphological features of the resting chromosomes are closely connected with the variation-pattern of species and the wide-crossability and phylogenetic interrelationships of species. The five taxa of the present Chenopodium showed a large difference within each other in the condition of inhabited environment, while taxonomically they were placed in close interrelationship. The
morphological features of the chromosomes of these taxa were found to be similar in both the resting stage and the mitotic stage. Considering these findings it can be proposed that the morphological features of the chromosomes appear not as a product of adaptive reaction to environmental conditions but as that of phylogenetic reaction.

It may be said that the morphological features of the chromosomes are produced by the functional reaction of chromosomes to the urgent physiological activity of the cells (Tanaka 1977). The differential features of the morphology of chromosomes among species suggest that the chromosomes of different species react to the same cellular function by differential morphological features. Comparative studies of karyotypes should be carried out considering this differential morphological reaction of the chromosomes.

According to the observations of the present Chenopodium and the previous Orchidaceae (Tanaka 1971a, 1971b), the morphological features of the chromosomes are a reliable indication of phylogenetic reaction in plants, and not an adaptive reaction to environmental conditions, thus confirming anew that the study of karyotypes is valid in the study of systematic evolution.

Summary

1. Karyomorphological studies were carried out in halophytic and non-halophytic Chenopodium.

2. Halophytic C. glaucum was diploid with n=9, 2n=18; semi-halophytic C. acuminatum var. japonicum was tetraploid with n=18, 2n=36, non-halophytic C. album was hexaploid with n=27, 2n=54, C. album var. centrorubrum hexaploid with 2n=54, and C. ambrosioides var. pubescens n=16, 2n=32.

3. The five taxa were found to have similar karyomorphological features categorized into the same ‘simple chromocenter type’ at resting stage, proximal and gradient heterochromatic type at prophase, and homogeneous type in size and symmetric type in arm-ratio at metaphase.

4. Morphological features of chromosomes were found to be correlated to the phylogenetic interrelationships in taxa, while no particular feature was found in relation to halophytic habit, except for polyploidy.

5. It was proposed that the morphological features of the chromosomes appear not as the product of adaptive reaction to the environmental conditions but as the product of phylogenetic reaction.

Literature cited


Hsu, C.-C. 1968. Preliminary chromosome studies on the vascular plants of Taiwan (II). Taiwania 14: 11–27.


