Morphological Observations on Capitulum Initiation and Floret Development of Garland Chrysanthemum (*Chrysanthemum coronarium* L.)

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Summary

Morphological properties of capitulum initiation and floret development of garland chrysanthemum (*Chrysanthemum coronarium* L.) were observed with a scanning electron microscope (SEM). Sixteen cultivars varying in flowering time were used. The flowering process was classified into 10 stages. A capitulum was initiated from stage 2 (early involucre forming-stage). The differentiation between ray and disc florets occurred at stage 5 (late floret forming-stage). Disc florets has a pistil and five stamens. Disc florets covered the entire surface of receptacle except for one or two layers of ray floret which are located on the periphery of the receptacle between the disc florets and the involucre. A ray florets has a pistil, but no stamen.

There were cultivar differences in capitulum size, numbers of ray and disc florets, and involucre. The diameter of the capitulum ranged from 71.0 ± 0.0 mm to 42.0 ± 6.2 mm; the number of ray florets per capitulum ranged from 23.7 ± 5.4 to 14.2 ± 0.5 ; where the range of disc florets was from 478.0 ± 0.0 to 276.2 ± 44.8 . The number of bracts (involucre) per capitulum ranged from 41.0 ± 0.0 to 27.50 ± 5.0 .

Individual cultivars differed in flowering response between spring and autumn. In the spring, budding in all cultivars occurred earlier than that in autumn. Capitulum of 'Kairyo chuba satoyutaka', 'Tatsunami', and 'Otafuku-A' became visible 10, 15, and 35 days after planting, respectively. In autumn, however, capitulum initiation of 'Kairyo chuba satoyutaka' occurred 70 days after planting.

The number of nodes from the cotyledon to capitulum in the spring was significantly fewer than that formed in the autumn. The node number of 'Kairyo chuba satoyutaka' was 32.8 in the spring, and 54.7 in the autumn.

Introduction

Garland chrysanthemum (*Chrysanthemum coronarium* L.) is an annual plant which originated in the Mediterranean region and introduced to other areas of Europe, Africa, and Asia. Its names are chop suey greens in North America and crown daisy in Europe (Bailey, 1949; Herklots, 1972; Larkcom, 1991; Park, 1980). It developed as a leafy vegetable in Japan, China, and South-East Asia. It is a popular leafy vegetable that is widely used in many traditional Japanese dishes.

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Studies on flowering and branching of garland chrysanthemum (*Chrysanthemum coronarium* L.) (1)

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The relation among earliness to flower, plant form, and leaf lobeness has not been investigated. Garland chrysanthemum is traditionally divided into three types, according to plant form and leaf lobeness. Shinohara (1984) reported that small leaf types are usually deeply lobed and flowers early. Clones with intermediate leaf size are characteristically moderately lobed and also flowers early. The large leaf types normally have shallow lobes and flowers late. Iwami et al. (1983) reported that flowering of garland chrysanthemum is promoted by long day, whereas Mochizuki and Hiraoka (1978) reported that continuous lighting also promoted an initiation of flower bud. These reports indicate that flower buds are induced under a long photoperiod but Javier (1990) reported that flowering was promoted by short days

in Taiwan. Taiwan is located in latitude of 25 °, where the daylength ranges from 10.3 ~ 13.4 hr. With such conflicting observations, the inductive daylength condition for flowering in garland chrysanthemum is questionable. Iwami et al. (1983) reported that the germinating seeds exposed to low temperature form a capitulum earlier than the unchilled control, but they used only one cultivar. Hence, the inductive condition necessary to promote early capitulum initiation in this species, with respect to temperature is still unknown.

There are a number of investigations on floral development in many species; *Apium graveolens* L. var. *rapaceum* (Mill.) DC. (Booij et al., 1992). *Tanacetum cinerariaefolium* L. (Brown and Menary, 1994), broccoli and cauliflower (Fujime, 1983), komatsuna and shun-giku (Iwami et al., 1983), Chinese vegetables (Kakibuchi, 1994) and Japanese radish (Sagwansupyakorn et al., 1985). However, there is only one morphological report on flower bud initiation and differentiation of garland chrysanthemum (Iwami et al., 1983). They showed a sketch of that flower bud, but did not explain the morphological properties of florets. Garland chrysanthemum has an inflorescence named capitulum (Yulian et al., 1994a) consisting of an involucre (fused bracts), one or two whorls of ray florets, and disc florets covering the rest of receptacle. However, the origin and the differentiation of the involucre, ray florets, and disc florets were not investigated morphologically. Hence, how cultivar and seasonal differences affect the flowering response in the species are still unknown.

In this study, the floral development of garland chrysanthemum, morphological characteristics of the capitulum, ray and disc florets using scanning electron microscope (SEM) were investigated. Furthermore, cultivar differences in capitulum size, numbers of ray florets, disc florets and involucre in plants grown in spring and autumn were also investigated.

Materials and Methods

Experiment 1 (Floral stages)

Seeds of garland chrysanthemum 'Kairyo chuba satoyutaka' (Sakata Seed Co.) were germinated on moist filter paper in a growth chamber (NK System Biotron Type LH-200-RD) provided with 14 hr daylength and rest at 23 °C on June 2, 1992.

Two days later the germinating seeds were sown in seeding boxes (28 cm × 52 cm) containing soil-sand-vermiculite (5:2:2 by volume). Seedlings with three to four unfolded leaves were transplanted to 9 cm diameter plastic pots and placed in a greenhouse under natural photoperiod and maintained at 20 °C. When seedlings had six to seven leaves, they were planted in the open field on July 1, spaced at 25 cm × 25 cm. After applying basal fertilizer IB-604 3.6 kg/row (N32, P20, K28 kg/10 a) and lime 1.8 kg/row (99.2 kg/10 a), the two rows, 4 m in length and 1.2 m width, were covered with plastic mulch; the plants were cultivated as usual.

Beginning on July 1, 12 plants were sampled at 7-day intervals, and their shoot tips fixed in formalin-acetic acid-alcohol (FAA solution). The shoot tips were dissected under a stereoscopic microscope and the number of leaf primordia, diameter of capitulum, and floral stages were identified. The following fixing procedure, developed by Fujime (1983), was used. The shoot tips were fixed in 4 % solution of glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at 5 °C for 24 hr. The samples were rinsed 3 times in the phosphate buffer and post fixed with 1.6 % osmium solution in the same buffer at 5 °C for 3 hr. The materials were dehydrated sequentially in the ethanol series, 15 %, 30 %, 50 %, 70 % for 30 minutes, and then in 85 % and 95 % for 1 hr each and 100 % for 24 hr. The alcohol was exchanged in series with 50 % and 100 % iso-amyl acetate solutions for 12 hr. The shoot tip samples were then dried in a Hitachi Critical Point Drier (HCP-1), mounted on SEM stubs with a drop of silver paste, sputtered with gold palladium in a RMC-eiko corp. ion coater, and photographed with on a Hitachi S-800 Scanning electron microscope at 5 ~ 8 kV accelerating voltage.

Experiment 2 (Cultivar difference)

Seeds of 16 cultivars (see Table 2) were germinated on moist filter paper in a growth chamber provided with 14 hr photoperiod and rest at 23 °C in May and September of 1991; and in April and September of 1992. Plants were grown in a greenhouse under natural photoperiod. Minimum air temperature was maintained at 20 °C. When the seedlings had six to seven leaves, they were planted in the field. The plots were fertilized as in

Exp. 1. Twelve plants of each cultivar were cultivated as usual and the date of budding and anthesis and number of nodes from cotyledon to the capitulum were recorded for the four plantings. Because budding and anthesis occurred in autumn which is uncommon, the experiment was stopped 70 days after planting. Shoot tips were sampled and observed under a light microscope.

Results and Discussion

Floral stages

The flowering process was classified into 10 stages. Vegetative stage is characterized by a conical shoot apex with its leaf primordia (Fig. 1-0). For other species of composite, *Tanacetum cinerariaefolium* L., as described by Brown and Menary (1994), a slightly concave apical shape may occur during vegetative growth, especially when the meristem slightly enlarges owing to the initiation of leaf primordia.

At the dome forming-stage (Fig. 1-1), the shoot apex forms a dome whose diameter increases steadily and becomes a highly arched, nearly round receptacle.

At this early involucre-forming stage (Fig. 1-2), the primordia of involucre scales (bract or modified leaf) are initiated at the periphery of the

dome; a first evidence that the capitulum is forming. 'Kariyo chuba satoyutaka' initiated a capitulum on July 29 (Fig. 2) when more than 50 % of the plants reached late involucre-forming stage. Each cultivar differ as to when flower bud or capitulum differentiation and anthesis occur. The present aim of commercial production of garland chrysanthemum in Japan is to provide a stable, constant supply throughout the year, especially in summer. Morishita et al. (1987) reported using 22 cultivars that their growth and development were variable by cropping seasons.

At this late involucre-forming stage (Fig. 1-3), the bract primordia (involucre) appears and elongate around the base of the receptacle. These involucre initials are located just above the last leaf, their arrangement is similar to that of the leaf primordia at vegetative stage but is more compact. Floret initials are not yet visible during this stage.

At the early floret-forming stage (Fig. 1-4), 2 ~ 3 layers of florets are differentiated on the base of the dome. Two types of florets: disc and ray florets appear but they can not be identified as such as this stage. The disc florets develop further (Fig. 3) with the acropetal appearance of the corolla and five stamen primordia. The corolla primordium of disc floret has a small lobe on the margin.

At this late floret-forming stage (Fig. 1-5), the

Fig. 1. Photomicrographs showing the floral stages of garland chrysanthemum. 0: Vegetative stage. 1: Dome-forming stage. 2: Early involucre-forming stage. 3: Late involucre-forming stage. 4: Early floret-forming stage. 5: Late floret-forming stage. 6: Early corolla-forming stage. 7: Intermediate corolla-forming stage. 8: Late corolla-forming stage. 9: Anthesis.

Date of sampling	Floral stages ^z									
	0	1	2	3	4	5	6	7	8	9
7. 1	⊗⊗⊗⊗	∞	○							
7. 8	∞∞	⊗⊗⊗⊗	∞∞							
7. 15		⊗⊗⊗⊗	∞		○					
7. 22		⊗⊗⊗⊗		∞∞		∞				
7. 29		∞∞∞		∞	∞∞∞	○				
8. 6						○	∞	∞	∞∞∞	∞

Fig. 2. Floral stages in 'Kairyo chuba satoyutaka' in spring of 1992.

^z: Refer to Fig. 1.

Fig. 3. Photomicrographs of developing of disc floret. a: Flattening of the disc floret dome. b: A primordium changes into concave shape. c: Initials of stamen primordia appear with the corolla. d: Elongation of stamens. e: The corolla initials elongate. f: Well-developed corolla (fused petal) primordia.

disc florets occupy more than half of the receptacle surface and can be distinguished from the ray florets. Disc florets are round or globular (Fig. 3a), whereas the ray florets are triangular and occupy the basal one two layers between the central disc florets and the involucre.

At the early corolla-forming stage (Fig. 1-6), the receptacle apex (florets) becomes slightly depressed (concave). The developing floret cover about 80 % of receptacle. The newly formed disc floret appears similar to that in Fig. 3. The older disc florets enlarge, remaining globular but be-

come cup-shape as corolla differentiation takes place. Five stamens are initiated inside the corolla. The individual petal initials appear as small lobes on the margin which elongates slightly.

In the intermediate corolla-forming stage (Fig. 1-7), the advanced florets with a lobed corollas occupy nearly half of receptacle.

The late corolla-forming stage (Fig. 1-8) is reached when the older basal florets have well-developed corollas and cover more than half of the receptacle; the remaining disc florets are still differentiating floral parts.

At anthesis, florets are fully developed; the petals of the ray florets expand fully (Fig. 1-9).

Cultivar differences on capitulum size, a number of ray and disc florets and involucre

There were cultivar differences in capitulum size, numbers of ray and disc florets and involucre (Table 1). The largest diameter of capitulum was 71.0 ± 0.0 mm in 'Kabuhari' and the smallest one was 42.0 ± 6.2 mm in 'Nantai chuba'. The number of ray florets ranged from 23.7 ± 5.4 in 'Oba-C' to 14.2 ± 0.5 in 'Chuba-C', whereas the disc florets ranged from 478.0 ± 0.0 in 'Oba-B' to 276.2 ± 44.8 in 'Kanto type kabuhari'. The largest number of bracts per involucre was 41.0 ± 0.0 in 'Oba-B' and the least was 27.5 ± 5.0 in 'Chuba-A'. In general, the number of ray floret per capitulum was half that of the bracts.

Cultivar differences in flowering response in spring and autumn

Based on the number of days between planting and budding and anthesis, there were cultivar differences in flowering response between in spring and autumn. The interval between planting and budding for the all cvs. was shorter than that of

autumn-grown seedlings. Capitulum of 'Kairyo chuba satoyutaka', 'Hakata kairyo chuba', 'Oedo', 'Chuba-A', 'Chuba-B', 'Obitsu', 'Yamabiko chuba', 'Chuba-C', 'Tatsunami', 'Kabuhari' and 'Oba' became visible 10 ~ 15 days after planting in the spring, whereas those of 'Kabuhari chuba', 'Kiwame chuba', 'Nantai chuba', 'Koasa' and 'Otafuku' became visible more than 30 days after planting (Table 2).

In the autumn planting, the capitulum initiation of 'Kairyo chuba satoyutaka', 'Hakata kairyo chuba', 'Oedo', 'Chuba-A', 'Chuba-B', 'Obitsu', 'Tatsunami' and 'Kabuhari chuba' occurred at 70 days after planting (Fig. 4), whereas the buds of 'Kairyo chuba satoyutaka', 'Hakata kairyo chuba', 'Oedo', 'Chuba-B', 'Tatsunami' at 70 days after planting were still at stage 3; 'Chuba-A' and 'Obitsu' were stage 2 and the capitulum differentiation in 'Kiwame chuba', 'Kabuhari', 'Otafuku-A' and 'Oba-A' had not occurred.

Node number from cotyledon to capitulum of all cultivars in the spring planting was less than that in the autumn planting, e.g. the number of nodes in 'Kairyo chuba satoyutaka' was only 32.8 in the spring (Table 2) but 54.7 in autumn (Fig. 4).

The differences in environmental factors in the

Table 1. Cultivar difference in floret, involucre number and size of capitulum in spring of 1995.

No. Cultivar ^z	No. of floret		No. of involucre	Capitulum diameter (mm)
	Disc floret	Ray floret		
1. Hakata kairyo chuba	321.2 $\pm$ 42.0	20.7 $\pm$ 0.5	36.7 $\pm$ 3.6	49.5 $\pm$ 8.2
2. Chuba-A	291.0 $\pm$ 53.7	18.5 $\pm$ 3.5	27.5 $\pm$ 5.0	45.5 $\pm$ 5.0
3. Chuba-B	432.0 $\pm$ 0.0	21.0 $\pm$ 0.0	40.0 $\pm$ 0.0	51.0 $\pm$ 0.0
4. Chuba-C	242.2 $\pm$ 8.6	14.2 $\pm$ 0.5	34.2 $\pm$ 1.6	49.6 $\pm$ 3.0
5. Tatsunami	313.0 $\pm$ 12.7	15.5 $\pm$ 0.7	31.0 $\pm$ 0.0	55.5 $\pm$ 6.4
6. Kiwame chuba	296.3 $\pm$ 85.4	15.5 $\pm$ 3.7	30.5 $\pm$ 7.1	60.3 $\pm$ 8.8
7. Nantai chuba	276.2 $\pm$ 27.6	20.2 $\pm$ 0.8	33.0 $\pm$ 1.4	42.0 $\pm$ 6.2
8. Kyouwa chuba	311.5 $\pm$ 28.9	16.5 $\pm$ 1.4	36.7 $\pm$ 1.0	58.2 $\pm$ 3.8
9. Kairyo chuba	336.0 $\pm$ 23.4	20.2 $\pm$ 1.0	38.2 $\pm$ 1.8	56.7 $\pm$ 3.8
10. Kabuhari	281.0 $\pm$ 0.0	20.0 $\pm$ 0.0	34.0 $\pm$ 0.0	71.0 $\pm$ 0.0
11. Kanto type kabuhari	276.2 $\pm$ 44.8	15.7 $\pm$ 3.1	32.5 $\pm$ 3.1	51.5 $\pm$ 5.1
12. Oba-A	299.7 $\pm$ 15.2	16.3 $\pm$ 1.6	32.5 $\pm$ 1.1	46.2 $\pm$ 3.8
13. Oba-B	478.0 $\pm$ 0.0	22.0 $\pm$ 0.0	41.0 $\pm$ 0.0	50.0 $\pm$ 0.0
14. Oba-C	338.5 $\pm$ 45.1	23.7 $\pm$ 5.4	38.7 $\pm$ 1.8	52.2 $\pm$ 7.4
15. Koji Oba (AVRDC)	303.5 $\pm$ 24.4	17.7 $\pm$ 2.7	30.5 $\pm$ 1.6	57.5 $\pm$ 2.6
16. Oba-D (China)	309.2 $\pm$ 23.4	19.2 $\pm$ 3.1	34.7 $\pm$ 4.4	53.7 $\pm$ 3.1

^z 1: Nakahara, 2: Hashimoto, 3: Sakata, 4: Tohoku, 5: Takii, 6: Sakata, 7: Tohoku, 8: Nakahara, 9: Nakahara, 10: Sakata, 11: Nakahara, 12: Futaba, 13: Takii, 14: Nakahara, 15: AVRDC, 16: China.

Table 2. Cultivar difference in budding date, date of anthesis and node number to capitulum in spring of 1992.

Cultivar	No. of budding	No. of anthesis	Date of budding	Date of anthesis	No. of nodes
Kairyo chuba satoyutaka	10	8	7/12	7/27	32.8
Hakata kairyo chuba	10	8	7/15	7/29	36.5
Oedo	11	11	7/15	7/30	42.1
Chuba-A	12	10	7/17	8/2	34.8
Chuba-B	9	3	7/19	7/29	54.0
Obitsu	6	8	7/20	8/6	41.5
Yamabiko chuba	8	7	7/20	8/2	40.7
Chuba-C	12	8	7/21	8/10	41.3
Tatsunami	11	9	7/22	8/9	45.3
Kabuhari chuba	4	1	8/4	—	48.3
Kiwame chuba	10	—	8/5	—	42.3
Nantai chuba	7	4	8/9	—	45.8
Koasa	8	3	8/9	—	48.1
Kabuhari	12	9	7/20	—	44.3
Otafuku-A	8	3	8/9	—	44.2
Oba-A	12	10	7/19	7/29	41.9

Cultivar ^u	Floral stages ^z										No. of node
	0	1	2	3	4	5	6	7	8	9	
Kairyo chuba											
satoyutaka		○	○○	○○○○○	○						54.7
Hakata kairyo chuba		○○	○○	○○○○	○○						59.0
Oedo		○		○○○○○	○						62.8
Chuba-A		○○○○	○○	○○○							78.5
Chuba-B		○○○○		○○○○							61.0
Obitsu		○○○○○○	○○	○○							75.8
Tatsunami		○	○○	○○○○	○						51.0
Kabuhari chuba		○○○○○○	○○	○							79.3
Kiwame chuba	○○○○○										-
Kabuhari	○○○○○○	○○									-
Otafuku-A	○○○○○	○	○	○○							-
Oba-A	○○○○○○	○○									-

Fig. 4. Cultivar difference in flower bud initiation and node number to capitulum at 70 days after planting in autumn of 1991.^z: Refer to Fig. 1.

field, such as daylength and fluctuations in day/night temperatures between spring and autumn may affect the budding date and number of node to capitulum. The mean air temperatures in spring of 1991 and 1992 were 14.3 °C and 17.7 °C, respectively, whereas in autumn they were 26.8 °C and 20.1 °C, respectively. The photo-

period in spring was about 12 hr 16 min, whereas it was 11 hr 39 min in the autumn. Therefore, budding was hastened at 14° to 18 °C under a 12-hr photoperiod, compared to 20° to 27 °C and under a shorter photoperiod. This tendency is in agreement with the findings of Iwami et al. (1983) and Mochizuki and Hiraoka (1978) and our pre-

vious investigation reported that capitulum initiation is promoted by low temperature under a long day condition (Yulian et al., 1994b, c).

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シュンギクの頭状花序形成ならびに小花発育の形態観察

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摘 要

シュンギク (*Chrysanthemum coronarium* L.) の頭状花序形成ならびに小花の発達における形態を走査型電子顕微鏡 (SEM) を用いて調査した。早晚性の異なる 16 品種を供試した。花芽発達段階は 10 段階に分けられた。頭状花序は総包分化前期に形成された。筒状花と舌状花との差異は小花形成後期から認められた。筒状花には 1 本の雌ずいと 5 本の雄ずいがあった。筒状花は花床の全表面に形成された。1~2 重の舌状花は筒状花と総包の間に位置していた。舌状花に雌ずいはあったが、雄ずいはなかった。

頭状花序の直径、筒状花ならびに舌状花と総包の数には品種間で差が認められた。頭状花序の最大直径は 71.0 ± 0.0 mm (株張), 最小直径は 42.0 ± 6.2 mm (男体中葉) であった。筒状花数と舌状花数の最大は、1 花序当たりそれぞれ 23.7 ± 5.4 (大葉-B) と

478.0 ± 0.0 (大葉-B) であった。筒状花数と舌状花数の最小は、1 花序当たりそれぞれ 14.2 ± 0.5 (中葉-C) と 276.2 ± 44.8 (関東タイプ株張) であった。総包数の最大は 41.0 ± 0.0 (大葉-B) であり、最小は 27.5 ± 5.0 (中葉-A) であった。

春季と秋季における開花反応には、それぞれの品種間で差が認められた。春季では、すべての品種が出らいいし、秋季に比べて早くなった。出らいいは定植後、早生種 '改良中葉さとゆたか' で 10 日、中生種 'たつなみ' で 15 日、晩生種 'お多福-A' で 35 日となった。秋季では、'改良中葉さとゆたか' は定植後 70 日で花芽を分化した。また、春季の着花節位は秋季に比べて顕著に低下した。'改良中葉さとゆたか' の着花節位は、春季では 32.8 であったが、秋季では 54.7 と高くなった。