Changes in Apical Morphology during Floral Initiation and Reproductive Development in Quinoa (Chenopodium quinoa Willd.)

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A numerical scale for identifying main apex morphological development stages from vegetative to open flower in quinoa (Chenopodium quinoa Willd.) has been developed, using SEM photographs and stereomicroscope observations. The scale accounts for the different patterns of development found in the two inflorescence types known in the species (glomerulate and amaranthiform). Eight stages are described for the glomerulate inflorescence, and seven for the amaranthiform inflorescence. Development of the apical meristem ends with the formation of an apical flower in the glomerulate type, and is interrupted by the appearance of a cap-like structure in the amaranthiform type inflorescence. This structure has not been observed in other Chenopodium species. A terminal flower is formed in all flower-bearing second-order axes in the glomerulate inflorescence; and the formation of a cap is repeated for the apical meristems of second-order axes in the amaranthiform inflorescence. Differentiation of axillary meristems progresses basipetally at a constant rate of 0.21 nodes °Cd⁻¹ (base temperature 6-4 °C) for the glomerulate inflorescence (variety Baer I) and in two stages for the amaranthiform inflorescence (variety Amarilla de Maranganí): an initial faster period with a rate of 0.28 nodes °Cd⁻¹ (base temperature 3.7 °C) in the upper nodes and a second, slower one, with a progression rate of 0.07 nodes °Cd⁻¹ in lower nodes. A description of the distribution of the grain-bearing glomeruli on the mature inflorescence is given.

Key words: Quinoa, Chenopodium quinoa, floral initiation, inflorescence ontogeny.

INTRODUCTION

Scales describing apical meristem ontogeny in transition to the reproductive phase are useful not only for understanding morphogenetic aspects of development, but also as a base for physiological research, breeding, and decision-taking concerning agricultural management. Chenopodium quinoa Willd. is a pseudocereal that has recently attracted attention as a commercial crop because of its nutritional qualities (protein content and amino acid balance characteristics) and adaptability to marginal agricultural conditions (Risi and Galwey, 1984). Originating in the Andean Region, it is cultivated from southern Colombia (Region of Nariño, latitude 2° N) to Southern Chile (Mt. Cochrane, latitude 47° S).

The species has been assigned to the Subsection Cellulata of the genus Chenopodium (Aellen and Just, 1943). The inflorescence of quinoa is a panicle with a principal axis, from which secondary and tertiary axes originate (Risi and Galwey, 1984). Two types of inflorescences have been described for quinoa: amaranthiform and glomerulate. In the amaranthiform type the glomeruli (short branches bearing a group of flowers or grains) are inserted directly on second order axes, while in the glomerulate type the glomeruli are inserted on third order axes (see diagrams of inflorescence types in Fig. 1).

Quinoa flowers lack petals and both pistillate and perfect forms exist. A perfect flower has five sepals, five anthers and a superior ovary from which two or three stigmatic branches emerge (Hunziker, 1943). Generally the perfect flowers are located at the distal end of the glomeruli and the pistillate ones at the proximal end (Gandarillas, 1979).

Gifford and Tepper (1961) have partially described the ontogeny of the Chenopodium album inflorescence on the basis of microscopic observations of apex sections. They identified the appearance of bud primordia in the axil of very young leaves as the first sign of transition from the vegetative to the reproductive stage. Some days later a primordial compound inflorescence is visible, consisting of a terminal conical apex with numerous subjacent primordial inflorescences, attached to the primary inflorescence axis and subtended by small linear bracts. The main apex first generates lateral inflorescence primordia and then develops into a terminal flower. In Chenopodium amaranticolor the elongation of the apex above the youngest leaf primordium has been identified as the first sign of floral initiation (Thomas, 1961) through stereomicroscopic observations of dissected specimens. This is followed by a sharp increase in the rate of initiation of bud primordia relative to that of leaf primordia, leading to a decreasing number of leaf primordia showing no axillary bud primordia. For quinoa, a sequence of development stages from vegetative stage to physiological maturity has been proposed by Jacobsen and Stolen (1993), based on the externally visible characteristics of the growing inflorescence. The above mentioned descriptions are not sufficient for a full understanding of the effects of temperature and photoperiod and description of the process of floral initiation plus inflorescence development. The des-
The objectives of this work were to describe the sequence of developmental stages of the main apex was established through stereomicroscopic observations of fresh material and SEM. Three plants were randomly sampled from each replication in both experiments. Floral stage was determined as the time when 50% of the plants sampled reached that stage. The patterns of development at both apex and inflorescence levels were expressed on a thermal time basis; computed as the accumulation of mean daily temperatures (maximum + minimum/2) over a base temperature (Ritchie and Ne Smith, 1991). Base temperatures were estimated for each variety by plotting the rate of development towards flowering (the inverse of the duration in days from emergence to flowering) vs. average temperature from the period (Summerfield et al., 1991), using data from another experiment which included seven sowing dates from Jul. 1992 to Feb. 1993. A base temperature of 6.4 °C was calculated for Baer I and 3.7 °C for Amarilla de Marangání.

At physiological maturity, five plants per inflorescence type were sampled for each replication in the 1995 experiment to analyse the distribution of the grain-bearing glomeruli on the inflorescence and number of grains glomerule.

**Table 1. Stages in development of the apical meristem of glomerulate and amaranthiform inflorescence types**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Glomerulate inflorescence</th>
<th>Stage</th>
<th>Amaranthiform inflorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>G0</td>
<td>vegetative</td>
<td>A0</td>
<td>vegetative</td>
</tr>
<tr>
<td>G1</td>
<td>early reproductive</td>
<td>A1</td>
<td>early reproductive</td>
</tr>
<tr>
<td>G2</td>
<td>exposed apical bud</td>
<td>A2</td>
<td>exposed apical bud</td>
</tr>
<tr>
<td>G3</td>
<td>beginning floral</td>
<td>A3</td>
<td>transition to cap stage</td>
</tr>
<tr>
<td>G4</td>
<td>beginning ovary formation</td>
<td>A4</td>
<td>cap stage</td>
</tr>
<tr>
<td>G5</td>
<td>ovary wall partly</td>
<td>A5</td>
<td>cap elongating</td>
</tr>
<tr>
<td></td>
<td>covering ovule</td>
<td></td>
<td>laterally</td>
</tr>
<tr>
<td>G6</td>
<td>ovule covered</td>
<td>A6</td>
<td>scar stage</td>
</tr>
<tr>
<td>G7</td>
<td>differentiation of</td>
<td>A7</td>
<td>anthesis</td>
</tr>
<tr>
<td></td>
<td>stigmatic branches</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G8</td>
<td>anthesis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Materials and Methods**

**Source of material and environmental conditions**

Two quinoa varieties: Baer I (glomerulate type panicle) and Amarilla de Marangání (amaranthiform type), were cultivated under optimal irrigation and nitrogen fertilization on a silty clay loam (Vertic Argiudol) soil at the Faculty of Agronomy, University of Buenos Aires, Argentina (34° 35' S, 58° 29' W) in a complete block design with two replications. Plant density was 30 plants m⁻². Data from two sowings (Jan. 1994, mean daily temperature 23.6 °C, mean photoperiod 13.8 h, mean total incident radiation 21.2 MJ m⁻² d⁻¹); and Feb. 1995, (mean daily temperature 20.8 °C, mean photoperiod 12.1 h, mean daily total incident radiation 15.3 MJ m⁻² d⁻¹) were used for these observations. Baer I is a cultivar originated in Southern Chile (Temuco, 38° 45' S, 72° 40' W), and Amarilla de Marangání originates from Cuzco, Peru (Cuzco, 13° 32' S, 71° 57' W).

**Specimen preparation**

Samples were taken every 2 d from emergence to flowering, dissected and observed through a stereomicroscope to determine floral stage on fresh material. To provide a graphical support to the description generated in this way part of the sampled specimens were subjected to Scanning Electron Microscopy (SEM). These specimens were fixed in FAA, dehydrated in a graded acetone series (70, 80, 90, 100%), critical-point dried, mounted on SEM stubs using conductive paint, dissected to expose the apical meristem and sputter-coated with gold/palladium.

**Data collection**

Two aspects of the pattern of floral development were studied. In the 1994 experiment the sequence of developmental stages of the main apex was established through stereomicroscopic observations of fresh material and SEM. In the 1995 experiment, the progression of development (as reflected by the achievement of a particular floral stage) from the apical meristem downwards through the inflorescence was followed using the scale previously established, through stereomicroscopic observations on fresh material. Three plants were randomly sampled from each replication in both experiments. Floral stage was determined as the time when 50% of the plants sampled reached that stage. The patterns of development at both apex and inflorescence levels were expressed on a thermal time basis; computed as the accumulation of mean daily temperatures (maximum + minimum/2) over a base temperature (Ritchie and Ne Smith, 1991). Base temperatures were estimated for each variety by plotting the rate of development towards flowering (the inverse of the duration in days from emergence to flowering) vs. average temperature from the period (Summerfield et al., 1991), using data from another experiment which included seven sowing dates from Jul. 1992 to Feb. 1993. A base temperature of 6.4 °C was calculated for Baer I and 3.7 °C for Amarilla de Marangání.

At physiological maturity, five plants per inflorescence type were sampled for each replication in the 1995 experiment to analyse the distribution of the grain-bearing glomeruli on the inflorescence and number of grains glomerule.
RESULTS

Apical meristem developmental scale

Eight floral stages were identified for the apical meristem in the glomerulate and seven for the amaranthiform inflorescence, as summarized in Table 1. In glomerulate inflorescences, apical meristem development ends with the formation of a terminal flower; in amaranthiform inflorescences development of a terminal flower is interrupted and diverted toward the formation of a cap structure. Flower formation in this inflorescence occurs only in lateral meristems.

Description of stages

1. Glomerulate inflorescence. Apical meristem development in this type of inflorescence progresses up to the formation of a terminal flower. In both apical and axillary buds, differentiation progresses basipetally. Axillary buds grow giving origin to second order axes, and an hermaphrodite flower is formed at the apical meristems of these ramifications. From these second-order axes third-order axes are formed. Flower-bearing glomeruli are supported by these third-order axes. The main stages of the apical meristem development observed in the glomerulate inflorescence are as follows.

G0. Vegetative: in this stage, the apical dome is...
hemispherical, and appears fully covered by leaf primordia. These primordia hide subjacent bud primordia (Fig. 2A).

G1. Early reproductive: the first sign of the transition towards flowering is an increase in the rate of growth of the apical meristem with respect to leaf primordia that leads to the emergence of the apical dome from among the leaf primordia (Fig. 2B).

G2. Exposed apex: the rate of axillary bud growth increases in relation to that of leaf primordia and thus become visible (Fig. 2C).

G3. Beginning of the differentiation of the terminal flower: the apex assumes a pentagonal shape owing to the appearance of five sepal primordia (Fig. 2D).

G4. Beginning of gynoecium differentiation: the apex expands to form a globose body, around which a rim soon develops (the future ovary wall) encircling a small dome (the future solitary ovule) (Fig. 2E). The sepal and stamen primordia are now clearly distinguished around the ovary.

G5. Ovary wall partially covering the ovular primordium, which in fresh material is distinguished by a more intense green colour (Fig. 2F). The two thecae of each stamen are clearly distinguishable.

G6. Ovary wall almost fully developed, ovular primordium no longer visible (Fig. 3A).

G7. Onset of differentiation of stigmatic branches. Four primordial stigmatic branches are apparent at this stage.

Fig. 3. Apical development in the glomerulate inflorescence; stages G6–G8. A, Ovary wall fully developed (G6); B, onset of differentiation of stigmatic branches (G7); C, pistillate flower (G8); D, perfect flower (G8); E, detail of pistillate flower showing rudimentary anthers. g, Gynoecium; t, thecae; ps, pollen sacs; ra, rudimentary anthers; s, sepals; sb, stigmatic branches; st, stamen. Bars = 0·1 mm.
Fig. 4. Apical development in the amaranthiform inflorescence. A, Vegetative (A0); B, early reproductive (A1); C, transition to cap stage (A3); D, cap stage (A4); E, cap elongating laterally (A5); F, scar stage (A6). a, Apex; ab, axillary buds; c, cap; d, dome; lp, leaf primordium; sc, scar. Bars = 0.1 mm.

(Fig. 3 B) and two pollen sacs are already differentiated in each theca. After the differentiation of stigmatic branches there follows a period of growth in size, elongation of stigmata and stamen pedicel, in parallel with pollen and ovule formation and maturation (events occurring in this period have not been quantified in this scale). This period ends at anthesis (G8), see below.

G8. Anthesis: defined as the time of the presentation of the anthers in perfect flowers and the elongation and emergence of the pistil in pistillate flowers. Pistillate and perfect flowers are shown (Fig. 3 C, D). Pistillate flowers are characterized by three very large stigmatic branches (one stigmatic branch is aborted) and no functional anthers (see rudimentary anthers in Fig. 3 E).

II. *Amaranthiform inflorescence*. Vegetative apices (stage A0) in this inflorescence are covered by leaf primordia as in glomerulate inflorescences, but are flatter and wider (Fig. 4 A). Stage A1 (early reproductive) is characterized by lateral displacement of the leaf primordia as a consequence of the increased lateral growth of the apex relative to leaf primordia (Fig. 4 B). After that, the apex forms a lobed, somewhat elongated rim, which appears to incorporate the last formed primordia (stage A4, Fig. 4 D). This cap-like structure usually shows a small dome at its centre. Transition to cap stage (stage A3) is shown in Fig. 4 C. After that, this cap extends laterally (stage A5, Fig. 4 E). Later growth of the surrounding area gradually reduces the cap-like structure to a centrally located, hidden scar or slit (stage A6, Fig. 4 F).
Since no terminal flower is generated in this type of inflorescence, the stage described as anthesis (stage A7) in Table 1 corresponds to the first open flower in the inflorescence.

Below the apical zone involved in the formation of the cap, axillary buds form secondary axes. Flower-bearing glomeruli are formed on these second-order ramifications, and the sequence of stages described for the main apex is repeated at the apical meristem of the ramification. Flower formation in the glomeruli follows the sequence described for the glomerulate inflorescence (Table 1).

**Rate of developmental progress**

**Apical meristem.** Apical development as quantified with the scale given in Table 1 progresses linearly as a function of thermal time between floral initiation and the last stage preceding anthesis in both inflorescence types (Fig. 5). The first stage of reproductive development at the apex (stage G1) occurred at 224 growing degree days (°Cd, base temperature 6-4 °C) from emergence in Baer I. Later stages followed at regular intervals of about 19 °Cd up to stage G7 (initiation of stigmata growth). A lag period of 180 °Cd was observed until stage G8 (anthesis). In Amarilla de Maranganı, the first floral stage occurred at 430 °Cd (base temperature 3-7 °C) from emergence, and an interval of 40-5 °Cd was computed for each stage between floral initiation and the scar stage. A period of 317 °Cd was computed between the scar and anthesis stages. Thermal times did not differ significantly between replications (P < 0.01) in both varieties.

**Axillary buds.** The stage chosen to describe downward progression of development of the inflorescence was stage G7 (differentiation of stigmatic branches) for the glomerulate inflorescence (Table 1). This is an easily distinguishable stage and allows an easy identification of node position of the flower primordia. For Baer I, the apical flowers of the second-order ramifications were observed; for Amarilla de Maranganı, the most advanced stage of flower primordia for the second order ramification (usually found near the base of the ramification) was used to define node stage. Differentiation progressed linearly at a rate of 0-21 nodes °Cd in Baer I up to nodes in lower positions into the inflorescence (Fig. 6A). Some of the lowest nodes did not form grains: ramifications in node positions lower than 15 senesced so they were not involved in grain formation. For Amarilla de Maranganı, progression showed a pattern with two rates: a first stage with a faster rate of progression (0-28 nodes °Cd⁻¹) and a second, slower one, (0-07 nodes °Cd⁻¹) beginning around node number 20 (Fig. 6B). In this variety, nodes below node number 31 scarcely contributed to grain formation, so the second phase of progression of development is not important in agronomical terms. Thermal time between differentiation of the first and last node was 212 °Cd for Baer I and 439 °Cd for Amarilla de Maranganı, equivalent to 16 and 27 d if compared using an average temperature of 20 °C. There were not significant differences between replications (P < 0.01) in both varieties.

**Distribution of glomeruli on the mature inflorescence**

In the glomerulate inflorescence the grain bearing glomeruli are supported by third order axes. The highest density of third order axes per second order axis, and of glomeruli per third order axis is found in subcentral positions into the inflorescence (Fig. 7A). The number of grains per glomerule (computed at the position of maximal number of glomeruli per third order axis) decreases exponentially from 8-6 grains per glomerule (s.e. = 0-52) in basal positions to 2 at top positions (s.e. = 0-09) (Fig. 7B). This results in the bulk of the grains being concentrated near the base of the inflorescence.

In the amaranthiform type the glomeruli are on second order axes, and the highest concentration of glomeruli per second order axis is also found near the base of the inflorescence, although in more central positions than in the glomerulate type (Fig. 7C). The number of grains per glomerule (computed at the position of maximal number of glomeruli per second order axis) decreases exponentially from 16-2 (s.e. = 2-2) at the base to 13 (s.e. = 0-2) near the top of the ramification (Fig. 7D). No differences were found between replications in their number of glomeruli per axis or grains per glomerule in both varieties (P < 0.01).
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Fig. 7. Number of glomeruli per axis and number of grains per glomerule for different positions on the inflorescence. A, Number of glomeruli per third-order axis as a function of third-order axis position on the glomerulate inflorescence. B, Number of grains per glomerule as a function of glomerule positions on third order axis in the glomerulate inflorescence. C, Number of glomeruli per second order axis as a function of its position on the amaranthiform inflorescence. D, Number of grains per glomerule as a function of glomerule position on the secondary order axis in the amaranthiform inflorescence. Positions are ordered from the basis to the top of the ramification. Vertical bars indicate ± 1 s.e. of the mean.

DISCUSSION

The descriptions of inflorescence development at the level of individual flowers and that of the whole inflorescence provide a useful framework for the study of reproductive development in this species. The initial floral stages in quinoa can be related to the ones observed in other Chenopodium species (Gifford and Tepper, 1961; Thomas, 1961). The appearance of the apex from among the leaf primordia (floral stage 1) is the first clearly distinctive signal of the transition toward reproductive development as observed in Chenopodium amaranticolor by Thomas (1961). The increased rate of axillary bud growth is observed later, in floral stage 2. This stage is equivalent to the one proposed by Gifford and Tepper (1961) as the beginning of reproductive development in Chenopodium album. The fact that the scale of Jacobsen and Stolen (1993) is based mainly on the quantification of changes in volume of the growing inflorescence, whereas the scale used here is based on a description of qualitative changes, makes it difficult to propose a correlation between the two scales other than for site-specific purposes. Stages three to seven in Baer I and three to six in Amarilla de Maranganí had not been documented before for any Chenopodium spp. Our results also show that the development of the two inflorescence types diverges shortly after floral initiation. The cap-like structure characteristic of the amaranthiform inflorescence seems unique to Chenopodium quinoa.

Briggs and Johnson (1979) adapted the Troll system (Troll, 1964) for the study of the evolution of the Myrtaceae. They characterized inflorescences as anthotelic (ending in a flower or in an aborted but distinctly floral bud) and blastotelic (not ending in a flower). The term anthotelic is equivalent to monotelic (Troll, 1964) and blastotelic to polytelic. Urmi-König (1981) used the terminology of Briggs and Johnson (1979) to describe flowering shoot systems of different Chenopodium species. They concluded that "some species show monotelic/polotelic (anthotelic/blastotelic) flexibility either in different individuals or of the second order axes of the same plant". For the species they analysed, none has a structure similar to the cap observed in Amarilla de Maranganí. Glomerulate inflorescences from quinoa can
clearly be assigned to the anauxotelic type. The truncate inflorescence of the amaranthiform type could be classified as an anauxotelic inflorescence, defined as a blastotetel inflorescence in which growth does not continue beyond the flowering region (Briggs and Johnson, 1979). This observation contradicts the assertion of Troll (1964, p. 177) that all the members of the Chenopodiaceae family have polietel (anauxotelic) inflorescences. Gifford and Tepper (1961) also observed terminal flowers in Chenopodium album.

Hunziker (1943) described pistillate flowers having two to three stigmatic branches, while we observed four stigmatic branches in the gynoecial primordium (stage 7). This apparent contradiction is solved when observing flowers at anthesis; only three stigmatic branches are present and one has been aborted.

There is an important variation between the two varieties in the time taken from the beginning to the end of floral differentiation for a given floral stage along the inflorescence. This is attributable to differences in total node number of the inflorescence and rate of progress through the inflorescence. Although there were differences in number of nodes (60 nodes in Amarilla de Marangani against 44 in Baer I, see Fig. 6) there were also variations in the rate of progress: Amarilla de Marangani progressed at a faster rate than Baer I in a first period, followed by a period of slower progression. In other crops there is also a linear progression of differentiation through different node positions. In soybean, flowering progressed acropetally as a linear function of thermal time, at a rate of 0.026 nodes °Cd−1 (base temperature 6 °C) (Munier-Jolain, Ney and Duthion, 1993); in wheat differentiation of floral organs progresses acropetally and basipetally along spikelets of different positions beginning in a sub-central spikelet (D. Miralles, pers. comm.). Comparing tall, dwarf and semidwarf genotypes of Argentine wheats, rates of progress for the stage of stamen and ovary primordia varied between 0.036 and 0.046 nodes °Cd−1 (basipetally) or between 0.069 and 0.084 nodes °Cd−1 (acropetally) (base temperature 0 °C). Using an average temperature of 20 °C to compare rates between species and cultivars with different base temperatures, the rate obtained for soybean is 0.36 nodes d−1, basipetal progress of development in wheat ranges from 0.71 to 0.81 nodes d−1 and acropetal progress from 1.38 to 1.68 nodes d−1. For quinoa, rate of progress for the glomerulate inflorescence is 2.86 nodes d−1; and 4.56 nodes d−1 for the first stage (faster rate of progression) of floral differentiation in the amaranthiform inflorescence. The second stage (slower rate of progression) has a rate of 1.14 nodes d−1. Comparing data from wheat and soybean with that of quinoa, the faster rate of progress of differentiation of this species is evident.

The scale here presented is easy to apply because its stages can be identified on fresh material with the aid of a stereomicroscope and it can be used as an effective tool to quantify environmental effects on the rate and duration of generation of floral organs in this species. Our observations also served to identify the origin of the differences between inflorescence types, and provide a first approach to the description of the distribution of reproductive structures on the inflorescence.

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LITERATURE CITED