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Article in *Australian Journal of Botany* · January 2009

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Floral morphogenesis and proliferation in *Poa labillardieri* (Poaceae)

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Abstract. Inflorescence and spikelet development in *Poa labillardieri* Steud. were investigated by scanning electron microscopy. Thirteen developmental stages were described in detail, starting with the vegetative shoot apex which was shown to be of the short type (stage zero), followed by a conversion from vegetative to floral meristem at Stage 1 and ending at Stage 12, with a mature panicle consisting of a variable number of florets at anthesis within each spikelet. The occurrence of short-type vegetative apices in this perennial grass adds further support to the view that there is no correlation between life span and the apex type. The branches of the *P. labillardieri* panicle are formed in acropetal succession; however, it is the upper branches that first bear rudiments of the spikelets, starting at the tip of the branches. In contrast differentiation of florets within each spikelet occurs in acropetal succession, so that the basal floret is farthest advanced and each successively upper floret less advanced. *P. labillardieri* occasionally produces inflorescences containing spikelets in which some or all of the florets are replaced by a plantlet that is structurally similar to a vegetative tiller. Proliferous development ranged from a situation where all florets were converted to vegetative propagules that can be detached and rooted readily in soil, to cases where proliferation extended only as far as an enlargement of the lemma, with either functional or non-functional sexual organs in its axil. Under greenhouse conditions, there was a shift from occasional cases of partially proliferating spikelets that occur in the wild to complete vigorous proliferation stimulated by unknown factor(s). Departure from the normal sexual pattern took place from early Stage 5 (initiation of spikelet primordial) to late Stage 6 (differentiation of florets).

Introduction

The grasses, Poaceae, constitute a large monocotyledonous family containing ~10 000 species (Kellogg 2000), including some of the most important food crops in the world. Because the characteristics of inflorescences and flowers are closely associated with traits for grain yield, developmental analysis has tended to focus on those species and their wild relatives (e.g. Liu *et al.* 1998). In addition, some of the most important pasture species, particularly *Lolium* spp., have been subject to developmental analysis to improve understanding of pasture production and seed-crop yield (e.g. Cooper 1951, 1964).

Detailed analysis of the floral development and embryology in the monocotyledons has assumed increasing importance in recent years in connection with systematic and phylogenetic studies (e.g. Rudall 1994 on the Xanthorrhoeaceae *sensu lato*) and the expression of sexual dimorphism (e.g. Caporali *et al.* 1994 with *Asparagus officinalis* and Ahmad *et al.* 2008 with *Lomandra longifolia*). However, within the grasses, such studies have been mainly confined to cultivated species, although there are several valuable exceptions (e.g. Le Roux and Kellogg 1999; Reinheimer *et al.* 2005; Sajo *et al.* 2007). Development studies of grasses other than the limited number of cultivated species make an important contribution to our understanding of the ecology, conservation and evolutionary biology of the grass family and have the advantage that the developmental sequences have not been subject to modification through the process of crop evolution

(Simmonds 1979). There is a complete absence of developmental information on the subject of our study, *Poa labillardieri*, a widespread native tussock grass in south-eastern Australia.

After a grass is induced to flower by conditions that are usually quite specific for a given species or ecotype, the vegetative meristem of a tiller becomes a floral meristem by a series of morphological changes that have often been described but are still poorly understood at the causal and organisational levels. The floral meristem then develops into an inflorescence that may vary in morphology from a panicle with several orders of branching to a spike with only short primary branches. These branches are terminated by a highly specialised shoot system, the spikelet, which consists of one to several florets. Molecular genetic studies, particularly in rice and maize, have identified several highly conserved genes which play a central role in these developmental processes (Bommert *et al.* 2005).

Conversely, development from floral to vegetative growth within the inflorescence has been recognised in many plant species. This phenomenon is known as proliferation or phyllody (Beetle 1980; Chapman and Peat 1992). Although the precise biological role of proliferation is not completely understood, it has been classified as pseudo-vivipary to distinguish it from true vivipary via sexual reproduction (Lee and Harmer 1980; Elmqvist and Cox 1996). Vegetative proliferation in grasses is defined as a development of shoot buds on the original spikelets of an inflorescence and has been

classified as a special asexual reproductive mechanism (Clayton 1990). More than 100 grass species have been reported to undergo vivipary, proliferation or phyllody (Beetle 1980; Roalson and Allred 1997; Miao *et al.* 1998).

Vegetative proliferation (pseudo-vivipary) is common in Festucoid grasses and has been studied in *Deschampsia*, *Festuca* and *Poa* species (Flovik 1938; Gustafsson 1946; Nygren 1949, 1967). Vickery (1970) stated that cases of proliferating spikelets 'have been found rather rarely' on some Australian *Poa* species, including *P. costiniana*, *P. hiemata*, *P. gunnii* and *P. labillardieri*. The first three species are confined to cool mountainous areas, but the last has a wider distribution from coastal to tableland areas. Pseudo-vivipary is a prominent feature of arctic and alpine species and may be an adaptive response to environmental stresses in adverse conditions (Lee and Harmer 1980). It is restricted to a limited number of species, indicating a genetic control, but as shown by several studies the expression of proliferation will only occur under the appropriate environmental conditions (Lawrence 1945; Nygren 1949; Langer and Ryle 1958; Youngner 1960; Latting 1966).

In the present study, we used scanning electron microscopy (SEM) to examine the sequence of events that give rise to normal inflorescence spikelets and florets of *P. labillardieri*, and compared these ontogenetic changes with the corresponding patterns in proliferated inflorescences.

Materials and methods

Source of material and specimen preparation for the morphogenesis study

Seeds of *P. labillardieri* were collected from a wild population at Tuggeranong, ACT (35°27'S, 149°07'E), in December 1999. Sixty-six seedlings were grown outside under sprinkler irrigation until maturity at Leppington Speedy® Seedlings and Supplies Pty Ltd, Leppington, New South Wales. The plants as supplied in July 2001 in 200-mm pots were trimmed at ~5 cm above soil level and cut into four portions, each portion being reset in a 125-mm pot using a potting mix consisting of nine parts

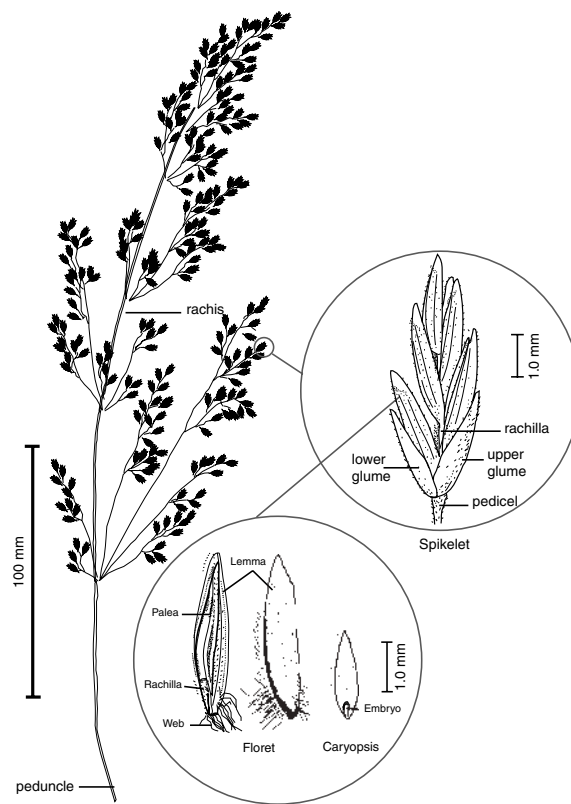


Fig. 1. An outline drawing of the panicle inflorescence of *Poa labillardieri*, showing a whole inflorescence and magnified views of a spikelet, florets and a caryopsis.

composted pine bark and one part washed river sand. The reset plants were left outside until the following winter, being subjected to the prevailing conditions of temperature and light at Cobbitty, New South Wales. Regularly watered and fertilised once a month, the plants tillered freely during the summer and after exposure to the low temperatures of May to mid-July 2002, they were

Table 1. Stages of inflorescence development in *Poa labillardieri* and their description

Thirteen-point scale based on Latting (1966), adopted to suit *Poa labillardieri*

Stage of development	Stage index
Vegetative	0
Early reproductive: elongation as the first morphological change	1
Double ridge	2
Primary branch primordia	3
Secondary branch primordia	4
Differentiation of spikelet primordia: appearance of glume primordia and budding of spikelet apex	5
Differentiation of florets: indicated by appearance of lemma primordia and floret apices	6
Differentiation of stamens, from first budding to lobed anthers; palea primordia appear at this stage. The floret apex develops differentially into an infolding ovary	7
Preboot stage: glumes enclosing most florets, anthers fully lobed, and protuberances appear on the upper surface of the infolding ovary	8
Early boot stage: glumes completely enclosing florets; when elongation of the panicle rachis has begun. Styles are tipped with stigma primordia	9
Boot stage: florets expanding above glumes; late boot stage, panicle and sheathing boot leaf elongating above vegetative leaves	10
Open panicle, preanthesis	11
Mature panicle, at anthesis; fertilisation, if any	12

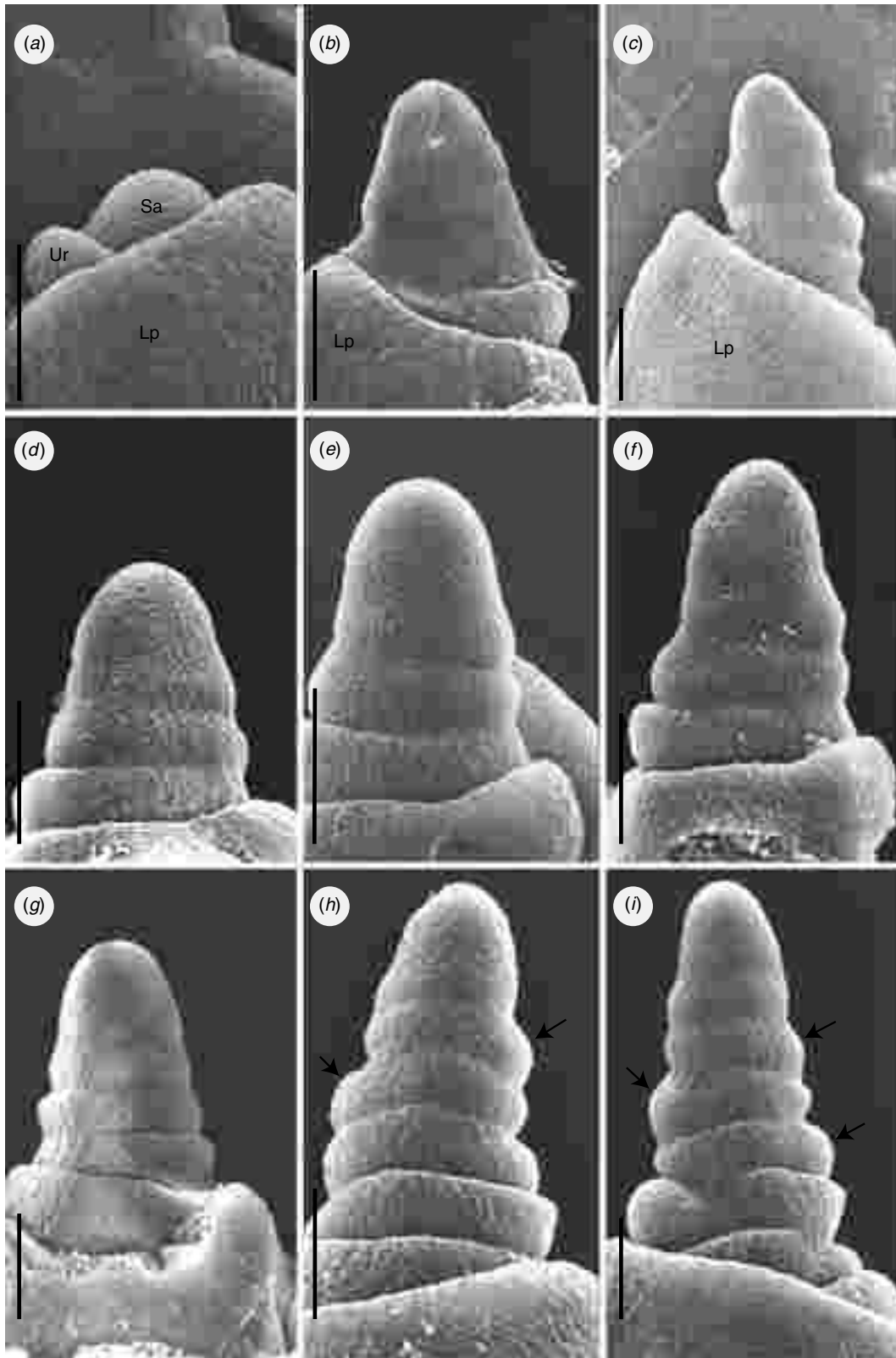


Fig. 2. Initiation and early development of an inflorescence in *Poa labillardieri*. (a) Stage 0, showing vegetative shoot apex with ensheathing leaf primordia. (b) Stage 1, transitional shoot apical meristem showing the elongation of the apex. (c–g) Stage 1, showing the formation of new ridges below the inflorescence apical meristem. (h, i) Stage 2, showing the appearance of swelling protuberances in the axils of leaf primordia (arrows). Scale bars = 0.1 mm. Lp, leaf primordium; Sa, shoot apex; Ur, upper ridge.

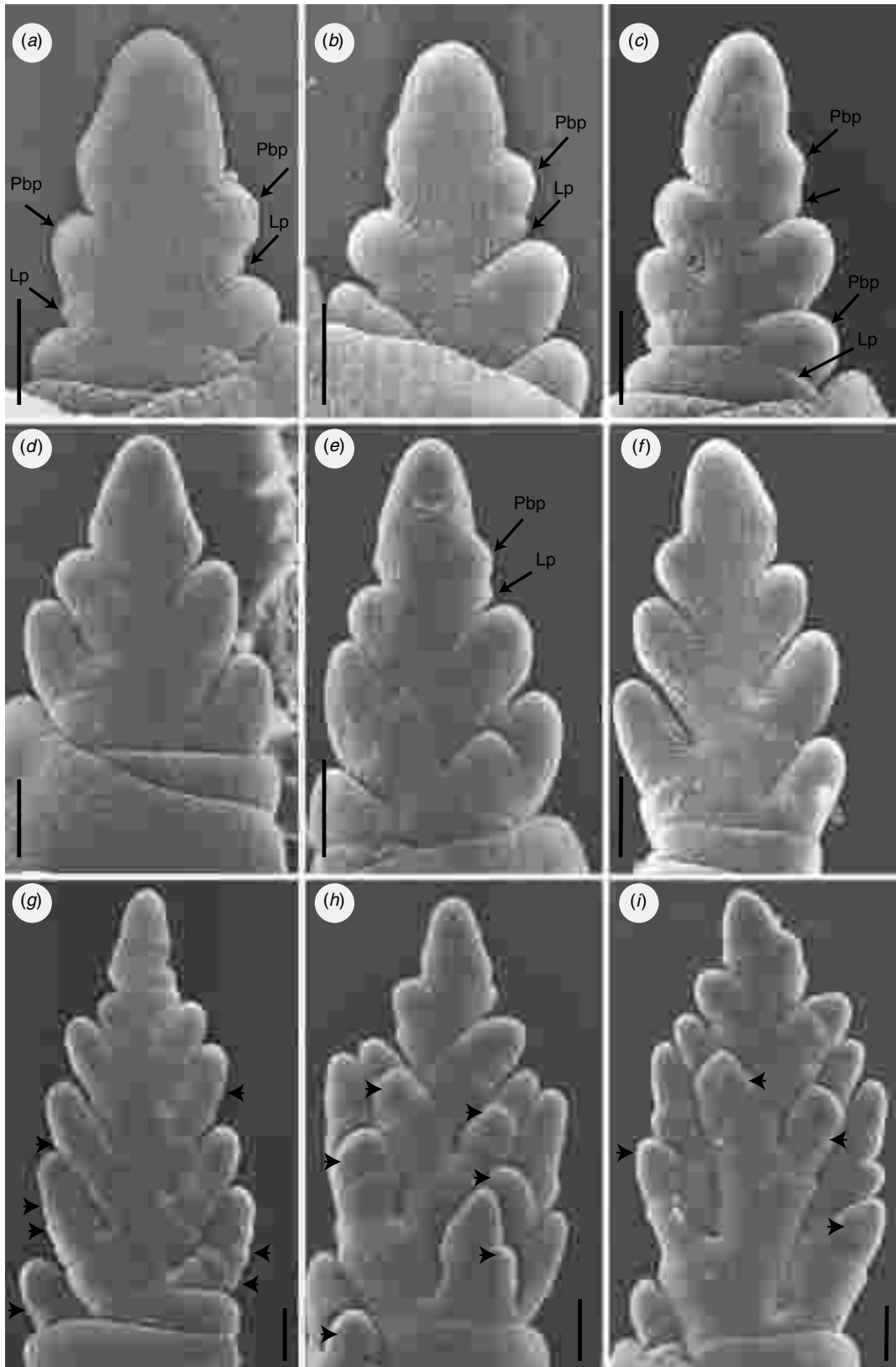


Fig. 3. Initiation of primary and secondary branch primordia in the developing *Poa labillardieri* panicle. (a–f) Stage 3, showing the initiation and differentiation of primary branches. (g–i) Stage 4, secondary lateral branch primordia arise on the primary branches (arrow heads). Scale bars = 0.1 mm. Lp, leaf primordium; Pbp, primary branch primordium.

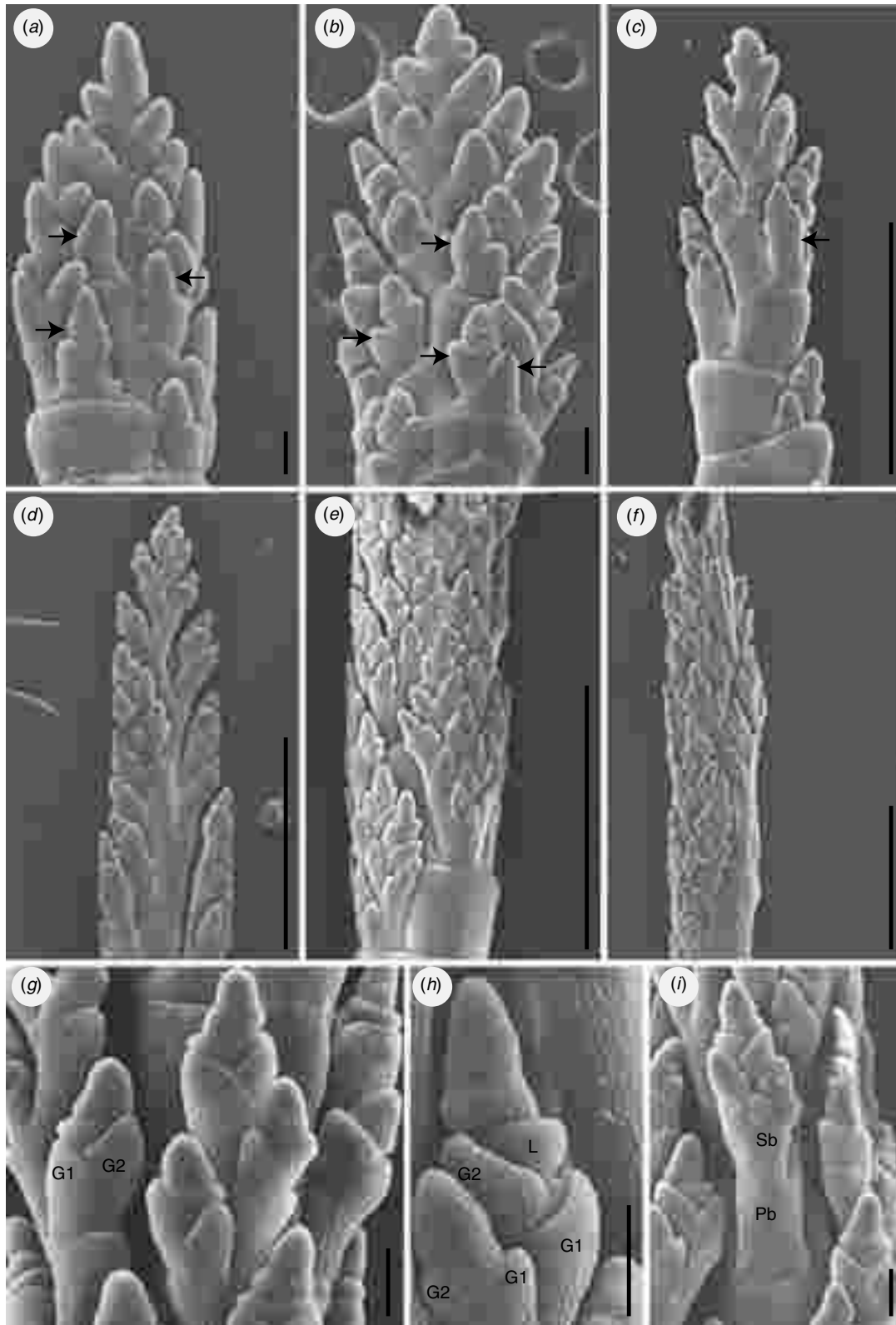


Fig. 4. Further secondary branch formation, spikelet differentiation and early floret differentiation in *Poa labillardieri*. (*a-c*) Late stage 4, showing secondary branch formation and elongation (arrows). (*d-i*) Stage 5, panicles are shown at the stage when the tip of the inflorescence and all lateral branches have been laid down and spikelet formation has just begun. They show rudiments of the glumes and, in some cases, of the lemma also. Scale bars = 0.1 mm (*a, b, g-i*) and 1.0 mm (*c-f*). G1, lower glume; G2, upper glume; L, lemma; Pb, primary branch; Sb, secondary branch.

transferred to a greenhouse and maintained at the prevailing uncontrolled temperature and light intensity where exertion of the panicle took place in mid-August 2002. Genotype 34 was selected for the developmental study and voucher specimens of that genotype were deposited in the Australian National Herbarium CANB (CANB 765300).

To describe the inflorescence of *P. labillardieri* and its development, shoot apical meristems at various developmental stages were dissected from reproductive stems of Genotype 34 under a stereo-microscope (ZEISS stemi 2000-c, Carl Zeiss, Göttingen, Germany) at several magnifications between $\times 6.5$ and $\times 50$. Dissected meristems at different developmental stages were fixed in 2.5% glutaraldehyde (in 0.1 M potassium phosphate buffer, pH 7.1) at 20°C for 2 h. After rinsing in the same buffer three times (5 min each time), they were dehydrated in a graded ethanol series (50, 70, 95 and 100%). Samples were critical point dried in CO₂ (BAL-TEC 030 critical point dryer, Bal-Tec, Liechtenstein) and mounted on sticky tape affixed to aluminium stubs after they were dissected under a stereo microscope with very fine forceps (number 5) and microneedles (250 μ m) to expose the apical meristems. Mounted specimens were coated with 20 nm gold-palladium in a sputter coater (Edwards E306 A, Edwards, Crawley, UK) and examined with a Philips 505 Scanning Electron Microscope (Philips, Eindhoven, Netherlands) operating at an accelerating voltage of 25 kV. Approximately 80 samples were examined at various stages of flower development, and representative stages were photographed with the installed digital camera.

Results

Normal inflorescence initiation and development

Poa labillardieri is a dense, tussocky perennial grass with slender leaves. Its paniculate inflorescences are 10–25 cm long, with erect or loosely spreading branches (Fig. 1), which in the case of the lower branches are devoid of spikelets for about half their length. Spikelets have three or four florets (rarely up to 8), greenish to purplish and strongly laterally compressed. Glumes are broad to rather narrow, subacute to occasionally subacuminate. Lemmas are 2.5–4.5 mm long, firm, narrow to moderately broad, usually hairy on the lower nerves, with a conspicuous basal web consisting of long hairs; palea firm and almost as long as lemma (Carolin and Tindale 1994; Wheeler *et al.* 2002).

The generally uniform pattern of early inflorescence development in grasses allows the use of a stage system based on changes that are readily distinguishable in the transition from vegetative to floral apex and subsequent development. The stage system given in Table 1 (adapted and modified from Latting 1966) represents different stages of initiation and differentiation that are numbered, starting from zero, denoting the vegetative stage, and progressing through a series to 12, the final number denoting the stage of the fully emerged inflorescence at the time of anthesis.

Description of stages

The main stages of shoot apex development observed in *P. labillardieri* were as follows:

Stage 0 (vegetative). The growing point of the shoot in *P. labillardieri* is very short, consisting of a rounded apex

with two ridges below, subtended by a basal leaf primordium which completely encloses the lower ridge and partially encloses the upper ridge (Fig. 2a).

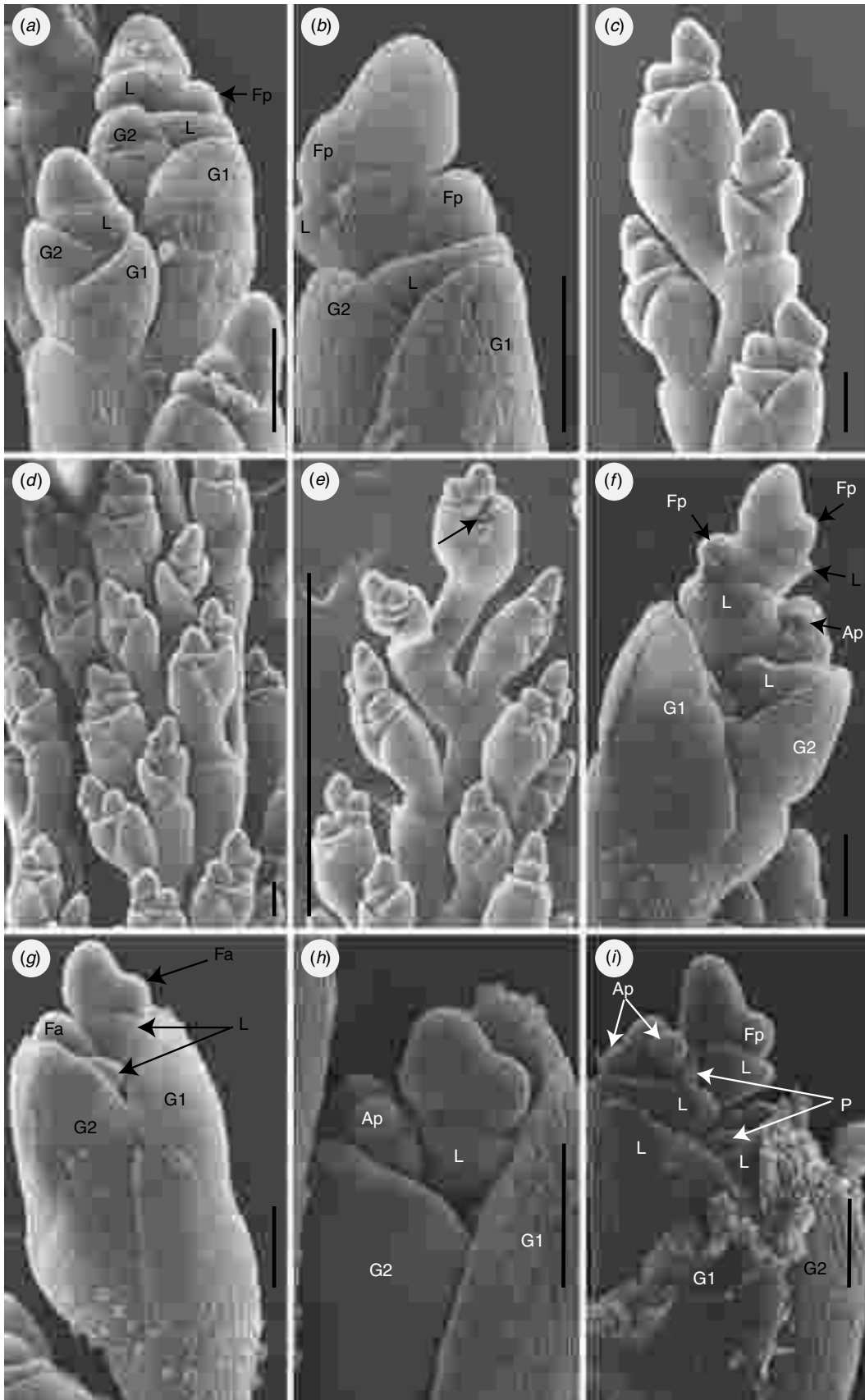
Stage 1 (early reproductive). The earliest morphological change in the apex indicating transition from vegetative to flowering state is elongation of the apex (Fig. 2b), owing to an increase in the rate of growth of the shoot apex with respect to leaf primordia. This leads to the emergence of the apical dome from among the leaf primordia, followed by the formation of new ridges below the rounded apex (Fig. 2c–g).

Stage 2 (double-ridge). Swelling protuberances appear in the axils of the leaf primordia (Fig. 2h, i). During their early development, these protuberances and their subtending leaf primordia have the general appearance of a double ridge at each incipient node. The first double ridges appear in the mid-region of the apex (Fig. 2h), with later protuberances appearing both distally and proximally (Fig. 2i). The apex at this stage is still elongating and initiating new ridges.

Stage 3 (primary branch primordia). The axillary protuberances of the double-ridge stage become further differentiated into primary branch primordia (Fig. 3a–f) because *P. labillardieri* has a paniculate inflorescence, whereas in grasses whose inflorescence is a spike or raceme, these protuberances differentiate directly into spikelet primordia. Further new leaf primordia and their axillary primary branch buds are continually being added, but now the buds develop so precociously that they and not the leaf primordia are the dominant features (Fig. 3f).

Stage 4 (secondary branch primordia). The young primary branches bear small ridge-like leaf initials, in whose axils arise secondary branch primordia (Figs 3g–i, 4a–c). These secondary branch primordia will bear leaf primordia which will subtend tertiary bud primordia. This repeated branching, seen in Fig. 4d, causes the inflorescences to take on the form of a panicle. The branches originating in the upper inflorescence, and formed last, remain small even in the mature panicle, forming a terminal compact cluster of branches. The lower and earlier-formed branches have a branch system which subdivides repeatedly, forming a compact mass that overlaps the branches above (Fig. 4d). The first-formed branches (at the lower part of the rachis) lag behind those formed later, so that, at the early stage shown in Fig. 4c, the largest branches are at about the middle of the panicle. The branches above the middle region are smaller now and subsequently in the mature panicle, whereas those below, although formed earlier and finally larger in the mature panicle, are currently smaller, developing at a slower rate the lower they are on the rachis. By the end of Stage 4, the inflorescence may reach a length of 2 mm.

Stage 5 (spikelet primordia). When the panicle is ~4 mm long (Fig. 4d–i), further elaboration is stopped by the tips of the uppermost branches beginning to become differentiated into spikelets by the formation of ridges which will develop into the glumes (Fig. 4d). The horns of the crescent-shaped rudiment of the first glume (lower glume) extend around the pedicel, but unlike the foliage leaf, the margins do not overlap. The second glume (upper glume) appears almost simultaneously with and very slightly above the first and below the apex of the branch, which thus becomes the rachilla (Fig. 4h). Differentiation of the spikelets takes place basipetally, not only



in the panicle as a whole but also in the individual branches of the inflorescence.

Stage 6 (differentiation of florets). Differentiation of florets begins with the appearance of lemma primordia and floret apices (Fig. 4*h, i*). Floret buds in the axil of each lemma differentiate in acropetal succession, so that in each individual spikelet, the basal floret is farthest advanced (Fig. 5*a–e*) and each successive upper floret less advanced. As a result, stamen development can be taking place in the basal floret even before the lemma has appeared in the upper one (Fig. 5*f*).

Stage 7 (differentiation of stamens and gynoecium). Stamen initials arise as rounded buds on the surface of the floret apex (Figs 5*f–i, 6a*). At the time when the anthers become bilobed, the floret apex appears as a spherical bud surrounded by the bilobed anthers (Fig. 6*b*). As the anther lobes become bilocular, the floret apex differentiates into a gynoecium which appears dimpled in the centre (doughnut shape) and later develops into an infolding ovary (Fig. 6*e, f*). At this stage, the developing anthers are at the same height as the infolding ovary, the lemma is about the length of the anthers and completely wraps around them. The glumes enclose most of the spikelet (Fig. 6*c–f*).

The palea arises from the floral axis above each lemma and just below the stamen initials (Figs 5*i, 6a*); it occupies the space between the bases of the two anterior stamens (Fig. 6*b*). The floret is oriented such that the palea is closest to the rachilla. The rudiments of the two lodicules appear at the same level as the palea, and occupy the space between the posterior and the two anterior stamens (note asterisk in Fig. 6*b*).

Stage 8 (preboot stage). Just before elongation of the inflorescence and after having three fully lobed anthers, two conical protuberances appear on the upper surface of the infolding ovary (Fig. 6*g, h*). Anthers at this stage keep growing and elongating, without any sign of filaments, to enclose the developing gynoecium.

Stage 9 (early boot stage). At the early boot stage, elongation of the panicle rachis has begun. Development of apical and mid-level spikelets and primary branch spikelets is advanced when compared with the lower and basal spikelets and secondary branch spikelets. Because of this, all stages of floret differentiation can be found simultaneously on the inflorescence at this time. Anthers of upper florets are lobed, those of lower florets budding around the floral apex. The two conical protuberances keep growing and elongating to develop into two linear styles placed laterally on the top of the ovary and tipped with primordia of the stigmatic papillae (Figs 6*i, 7a–f, h*). Anthers increase in length at the same time, being about two to three times the length of the lateral styles throughout this stage. Filaments become identifiable after the appearance of the stigma primordia (Fig. 7*d, e*) and elongate up to ~0.2–0.3 mm by the end of this stage (Fig. 7*h*). The glumes expand upwards to enclose all florets (Fig. 7*i*).

The bases of the two lodicules become fused so that the mature lodicules appear as a single bifid organ, and their margins become fused to those of the palea, forming a very short cylindrical sheath around the floral axis (Fig. 7*i*).

The internode (peduncle) between the base of the panicle and the uppermost leaf remains minute until an advanced stage in the development of the panicle. As the spikelets become more highly developed, the branches that bear them elongate (Fig. 7*g*), together with the rachis, until the entire protecting leaf-sheath is filled and the upper and more advanced spikelets protrude. The lowest branches are still only slightly differentiated, but as the top of the panicle slowly protrudes, their development is completed.

Stage 10 (boot stage). Further development of the gynoecium is shown by extensive growth with differentiation of a gynophore beneath the ovary, with branching of the stigmatic papillae and swelling of the ovary indicating an internal activity represented by ovule development (Fig. 8*a*). During the boot stage, further elongation of the panicle axis takes place concurrently, with florets expanding above the glumes. The peduncle elongates rapidly, pushing the whole panicle free of the protective sheath, marking the end of the boot stage while the branches that have remained in a tight bundle now spread out to form an open panicle.

Stage 11 (preanthesis). Stigmas elongate and become feathery in appearance (Fig. 8*b*). Anthers, green at this stage, become yellow or purple (according to genotype) as pollen ripens. Filaments of the stamens do not elongate until a very late stage, just before anthesis. During the late boot stage and panicle emergence from the sheath of the flag (boot) leaf, glume expansion ceases and florets expand above them (Fig. 8*c*).

Stage 12 (anthesis). At anthesis, lodicules swell, forcing apart the lemma and palea; and the stamen filaments elongate within 10–15 min around midday. Anthers become exerted and stigmas spread apart towards both sides of the floret (Fig. 8*d–i*).

Spikelet proliferation

Normal inflorescence development leads to an open panicle in which each floret includes functional sex organs (Fig. 9*a, b*). Proliferous development ranged from a situation where all florets were converted to vegetative propagules (Fig. 9*c*) that can be detached and rooted readily in soil (Fig. 9*d*), to cases where proliferation extended only as far as an enlargement of the lemma with either functional (Fig. 9*e, f*) or non-functional (Fig. 9*g–i*) sexual organs in its axil. Sometimes proliferation was confined to the elongation of the rachilla within the axil of the lowest lemma (Fig. 10*a*). In some cases, all three types of spikelets, together with the usual sexual spikelets, can be found in the same inflorescence (Fig. 10*a, b*), or the entire inflorescence has all its florets proliferated (Fig. 10*d*). Where proliferation led to the formation of vegetative propagules, the timing of the departure

Fig. 5. Floret differentiation in *Poa labillardieri*. (*a–c*) Stage 6, floret primordia in the axil of each lemma of the apical spikelets become well differentiated. Note in (*a, b*) floret differentiation occurs acropetally within each spikelet. (*d, e*) Late Stage 6, showing three floret buds of the apical spikelet appear as rounded protuberances in the axils of the lemmas. (*f–i*) Early Stage 7, spikelets with small and rounded anthers budding on older florets (black arrows). Primary spikelets have elongating glumes; their basal florets have prominent anther buds, but anther buds have not yet arisen on the distal florets which appear as a rounded protuberance in the axils of the flat lemmas; on the spikelet in (*f*) basal glumes and two lemmas with axillary floret buds are obvious, lemma and floret bud of the next distal floret appear as slight bulges on the upper right of apical dome. Scale bars = 0.1 mm (*a–d, f–i*) and 1.0 mm (*e*). Ap, anther primordium; Fa, floret apex; Fp, floret primordium; G1, lower glume; G2, upper glume; L, lemma; P, palea.

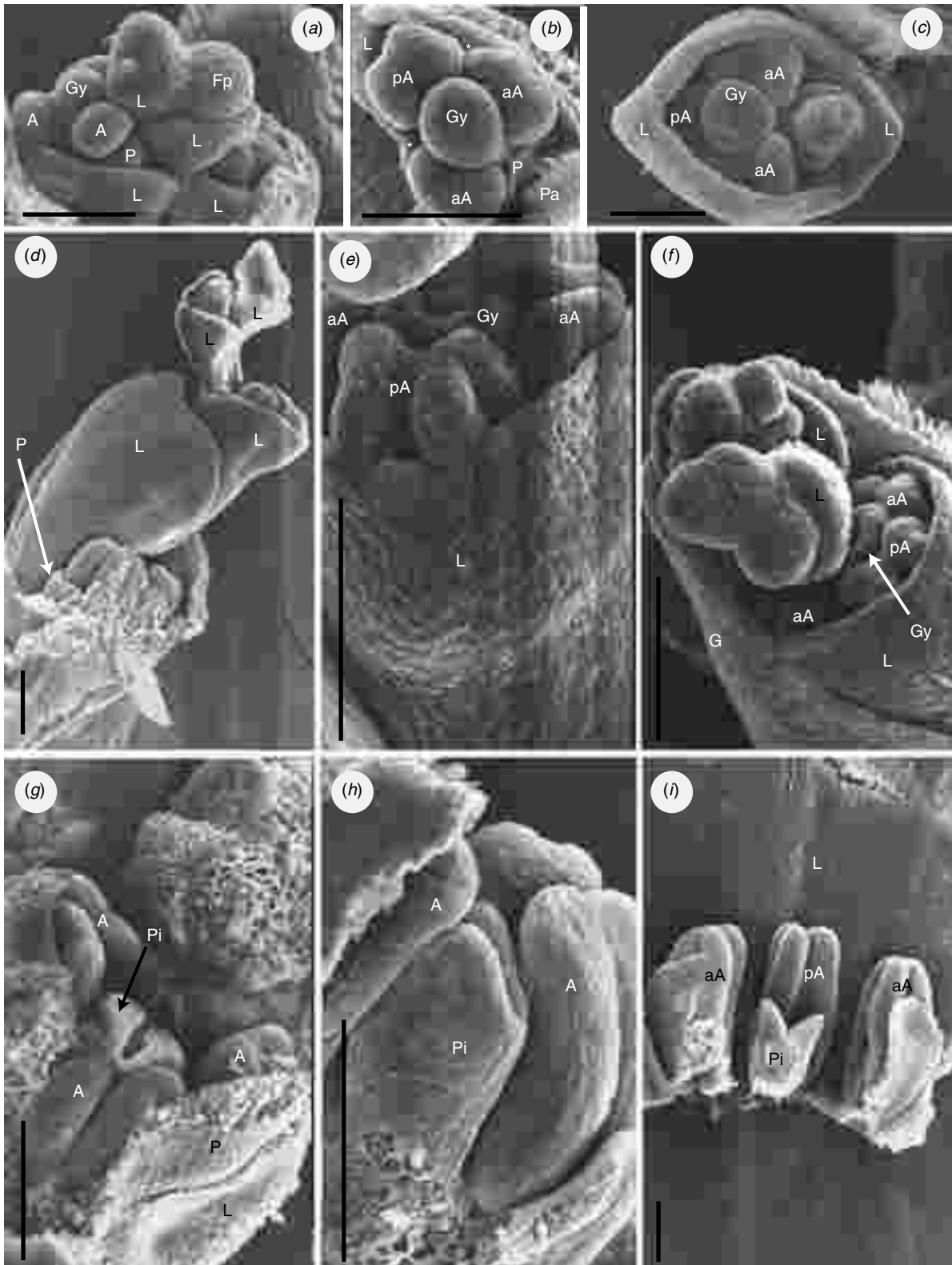


Fig. 6. Stamen and pistil differentiation in *Poa labillardieri*. (a–f) Late Stage 7, showing the floret apex of older florets differentiating into a gynoecium and three lobed anthers. Lodicules indicated by asterisks in (b) occupy the space between the posterior and the two anterior stamens. (g, h) Stage 8, showing two conical protuberances on the upper surface of the infolding ovary (arrow) and three fully lobed anthers. (i) Stage 9, showing further growth of the gynoecium. Scale bars = 0.1 mm. aA, anterior anther; pA, posterior anther; Fp, floret primordium; G, glumes; Gy, gynoecium; L, lemma; P, palea; Pi, pistil; Ra, rachilla.

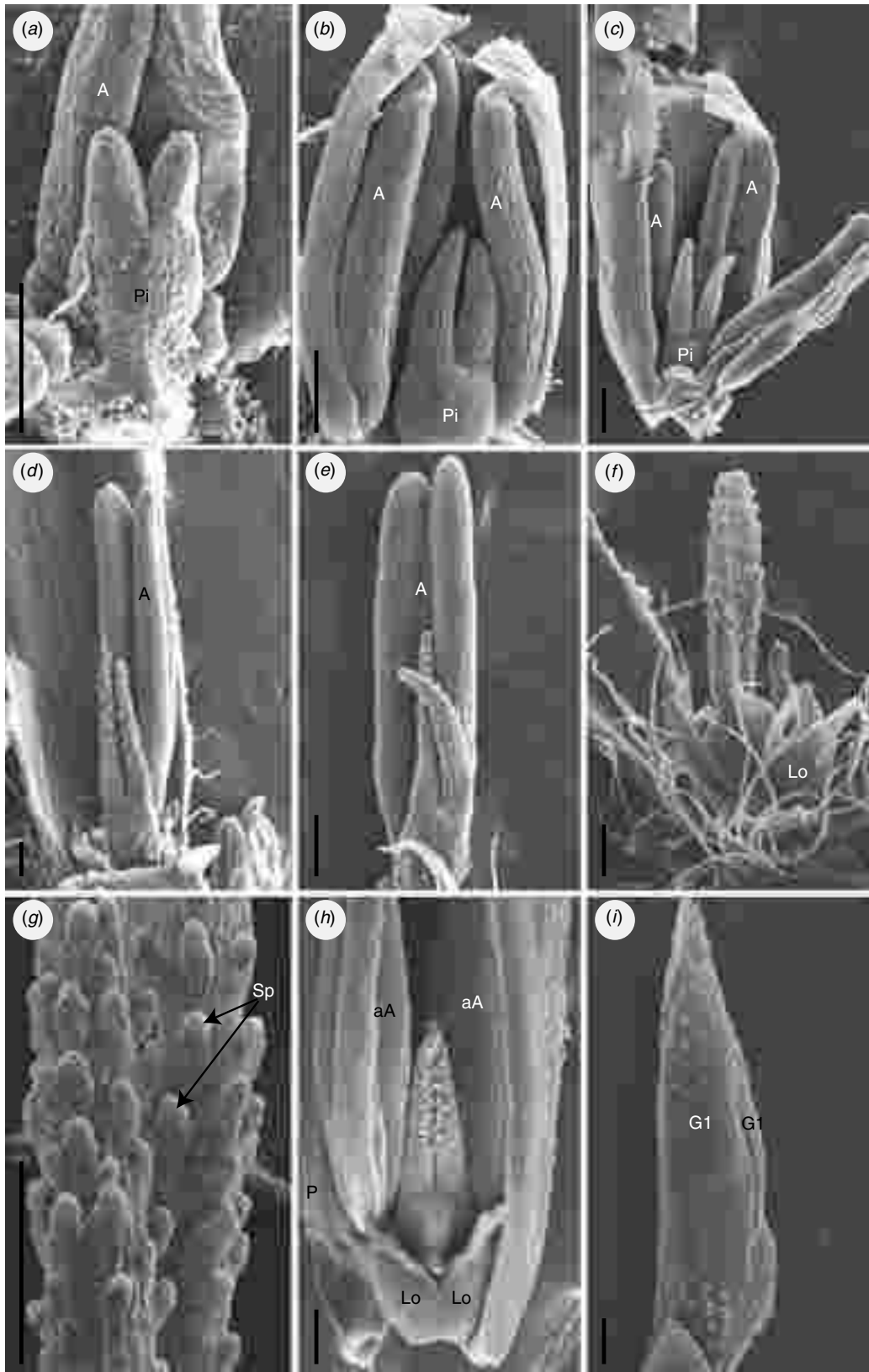


Fig. 7. Later differentiation of florets in *Poa labillardieri* at Stage 9, showing the growth of the gynoecium and the formation of stigma primordia (arrows), note the glumes expand upward to enclose all florets (*i*). Scale bars = 0.1 mm. A, anther; aA, anterior anther; G1, lower glume; G2, upper glume; Lo, lodicules; P, palea; Pi, pistil; Sp, stigma primordium.

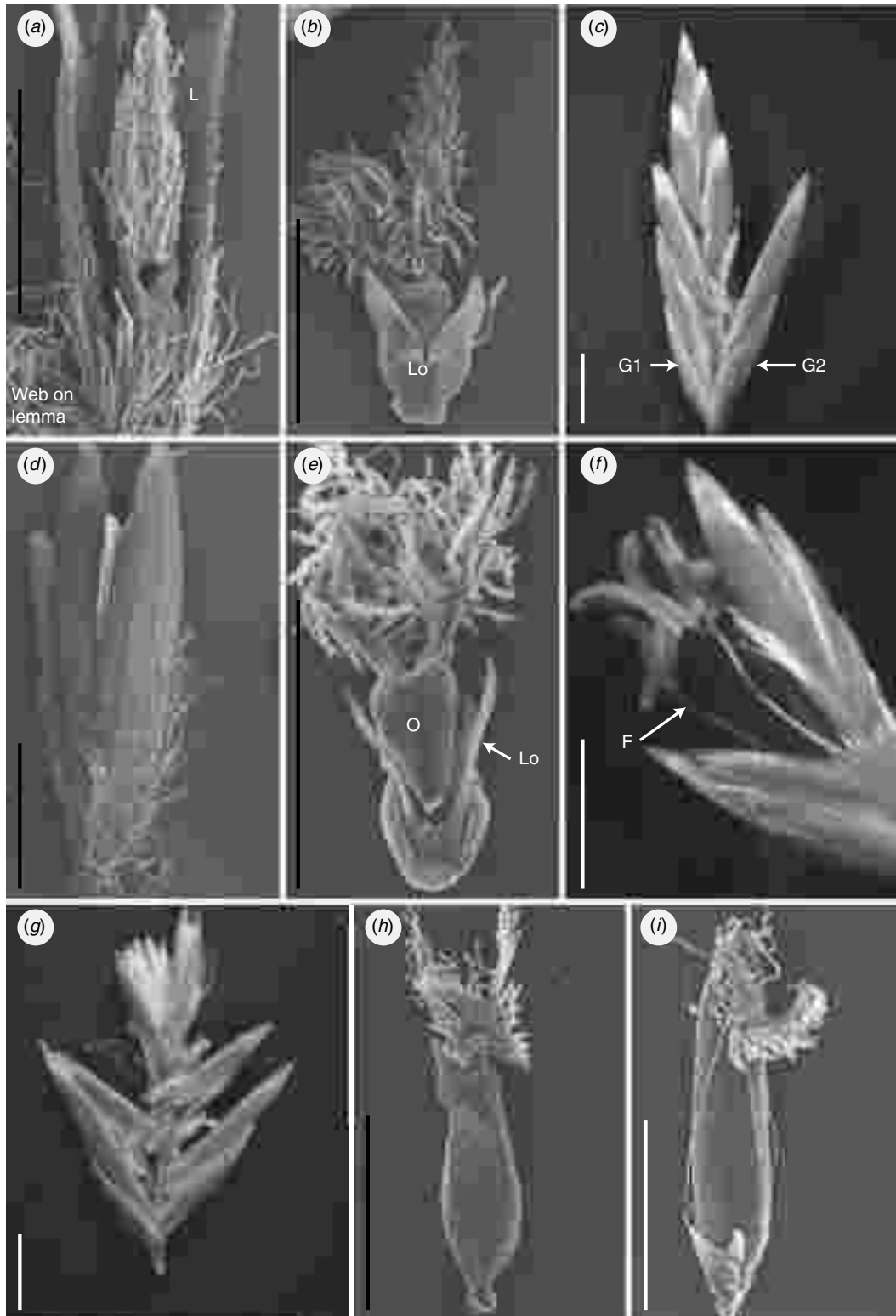


Fig. 8. Floret maturation, anthesis and post-fertilisation in *Poa labillardieri*. (a) Stage 10, showing further growth of the gynoecium and florets starting to expand above glumes. (b, c) Stage 11, showing the feathery stigma and the florets expand above the glumes. (d) Late Stage 11, showing a mature floret. (e–i) Stage 12, showing a mature gynoecium at anthesis in (e). (f) Shows a spikelet shortly after anthesis with filament elongation and shedding of pollen grains. (g) Is a spikelet showing acropetal maturation of florets where the lower florets have been fertilised while the upper floret is still at pre-anthesis stage. (h, i) Show fertilised florets (seeds) at different developmental stages. Scale bars = 1.0 mm. F, filament; G1, lower glume; G2, upper glume; L, lemma; Lo, lodicules; O, ovary.

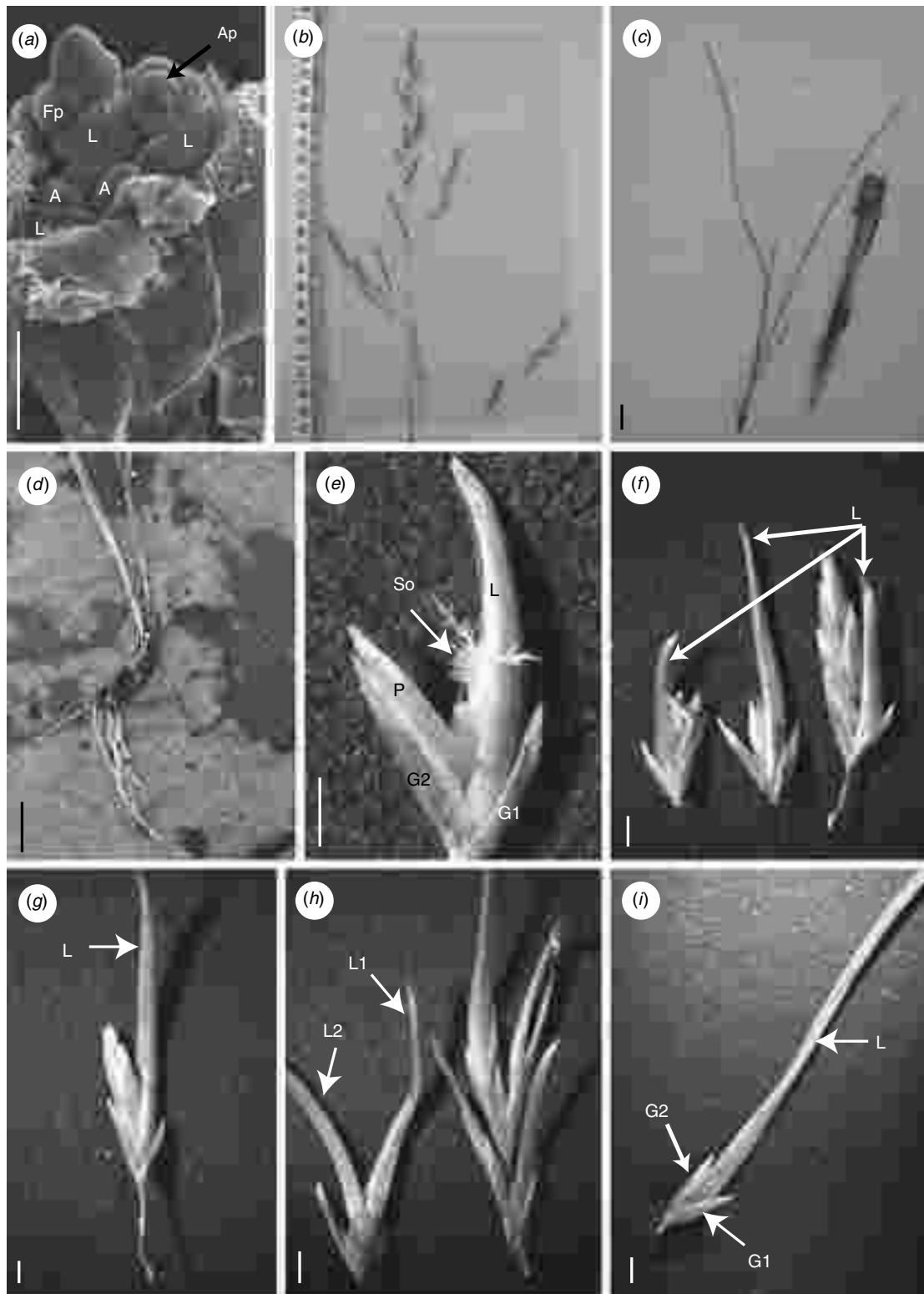
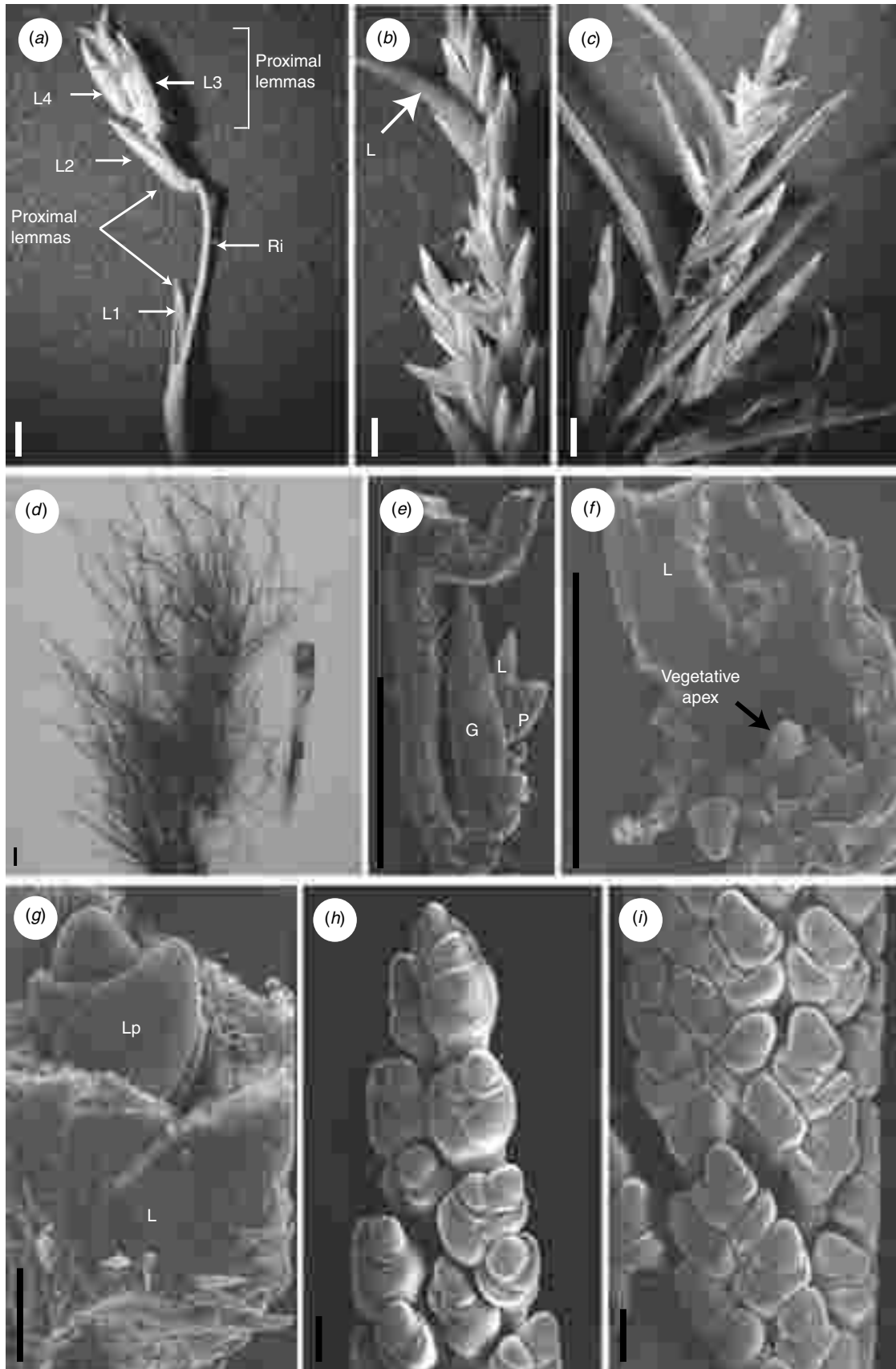


Fig. 9. Sexual (*a, b*) and proliferated (*c–i*) spikelets in *Poa labillardieri*. (*a*) Show normal inflorescence development which leads to an open panicle (*b*) in which each floret includes functional sex organs. Proliferated spikelets of *P. labillardieri* showing a situation where all florets were converted to vegetative propagules (*c*) which can be detached and rooted readily in soil (*d*) or as in (*e, f*) where each spikelet has a single enlarged lemma with functional floral structures in its axil and in (*g–i*) spikelets with enlarged lemmas as leafy shoot with no trace of floral organs in their axils. Scale bars = 0.1 mm (*a*), 15.0 mm (*c, d*) and 1.0 mm (*e–i*). A, anther; Ap, anther primordium; Fp, floret primordium; G1, lower glume; G2, upper glume; L, lemma; L1, first lemma; L2, second lemma; p, palea; So, sexual organs.



from the normal sexual pattern ranged from early Stage 5 to late Stage 6. In some cases, reversion to the vegetative state within a floret occurred at a very early stage of development (Fig. 10*e, f*), such that no trace of staminal or gynoecial organs could be seen; instead, the central region of the floret was occupied by a well defined vegetative apex similar in appearance to the normal vegetative apex shown in Fig. 2*b*. This departure from the sexual pattern of development takes place at about Stage 5. However, in contrast to the normal Stage 5 where the lemma remained very short, the lemma of the proliferating floret had already elongated so much that, as shown in Fig. 10*f*, it had to be cut short to facilitate the preparation of the specimen for SEM examination. Figure 10*g* is a closer view of the vegetative apex inside another proliferating floret. Figure 10*h, i* shows a whole inflorescence (~4 mm long) proliferating at a stage comparable with late Stage 6 in Fig. 5*d, e*. Here, the proliferating inflorescence shows no sign of anther initiation, and this is apparent in the more advanced florets at the top of the rachis as shown by the arrows in Fig. 5*e*.

Discussion

Normal inflorescence initiation and floral development

Scanning electron micrographs were used to prepare an analytical description of inflorescence and floral development in *P. labillardieri*. The overall developmental pattern is summarised in Fig. 11.

The vegetative apices of *P. labillardieri* are designated as the 'short type', according to Sharman's classification of apices in the grass family (Sharman 1947). This type of apex is also found in *Avena*, *Oryza*, *Saccharum*, *Secale*, *Sorghum*, *Triticum* and *Zea*, and seems to be common in the cereal grasses. However, as Sharman (1947) points out, the suggestion that short vegetative apices are characteristic of annuals and long ones of perennials is invalidated by the occurrence of short apices in *Phyllostachys* and *Saccharum* and of the intermediate and long types, respectively, in the annuals *Poa annua* and *Lolium multiflorum*. The occurrence of short apices in the perennial *P. labillardieri*, as revealed in the present study, adds further support to the view that there is no correlation between life span and the type of apex.

The branches of the inflorescence (Fig. 4*a, b*) are formed acropetally, although it is the upper branches that first bear rudiments of the spikelets, starting at the tip of the branches. The retardation of the development of the lower branches is such that at the time when the florets on the upper branches have reached a high degree of differentiation, those on the lower branches have barely commenced. At panicle exertion (Stage 10), the spikelets on the upper branches are the first to leave the protective leaf sheath, and they retain their advanced condition until the time of anthesis.

The development of an individual spikelet is initiated by the appearance of the crescentic rudiments of the two glumes which form almost simultaneously, the latter to appear being slightly higher on the pedicel. The horns of the crescents extend around the axis as the rudiments elongate but they do not completely encircle the axis nor do they fuse together.

The lemma appears very early, but develops slowly and does not enclose the anther rudiments until these are well developed with four lobes, when it is about the length of the anthers and completely wraps about them. The rudiment of the lemma encircles the rachilla more completely than those of the glumes; however, like them, its margins do not fuse.

The palea, presumably the equivalent of a prophyll on the axis of the floret bud in the axil of the lemma, is not visible in the SEM micrographs until the stamens have developed into spherical swellings below the floret apex (early Stage 7, Fig. 5*i*). It occupies the space between the bases of the two anterior stamens (Fig. 6*b*); however, no clue was found in the SEM images about the stage when it was initiated. The palea belongs to the whorl external to the stamens and would therefore be expected to initiate before them. However, if the palea grew slowly at first relative to the stamens it would be overshadowed by them and difficult to see, which could explain its seemingly late arrival. This suggestion gains support from Williams (1975, p. 195) who reported that in wheat (*Triticum aestivum*), in comparison with the anthers and ovary, the palea exhibits an early period of relatively slow growth which Williams (1975) linked to the overcrowding typical of this stage of spikelet development. Histological examination of serial sections of embedded material would be needed to clarify this point.

The stamens form very early as three spherical swellings below the apex of the flowering axis, which later becomes transformed into the pistil. The stamens become oblong and furrowed with the formation of the anther lobes. Until they are about half their mature length, the anthers are sessile on the floral axis, below the ovary; however, when the stigma primordia appear, the filaments begin to elongate, pushing the anthers towards the apex of the lemma. At the time of flowering, the filaments elongate further and the stamens protrude freely from the gaping glumes and lemma. By the time the stamen filaments are ready to begin elongating, the ovary is a heart-shaped structure, with two short, simple, rod-like style rudiments. Very little further change takes place before fertilisation; the ovary becomes more swollen with the development of the ovule, and the styles develop a much-branched stigmatic surface. After fertilisation, the ovary becomes cylindrical but remains topped with the branched feathery stigma until very late stages of seed development.

In summary, the normal *P. labillardieri* spikelet consists of a main axis, or reduced shoot, bearing the glumes at the base, with

Fig. 10. Abnormal inflorescence development in *Poa labillardieri* showing in (a) elongated rachilla with no trace of sexual organs in the axils of the proximal lemmas, while the distal florets have elongated lemmas with sexual organs in their axils, in (b, c) is an inflorescence with partially proliferated spikelets. (d) Is an inflorescence with completely proliferated spikelets. (e–i) Scanning electron micrographs (SEM) showing completely proliferated spikelets in which the lemma of the proliferating floret had already elongated, so much that as shown in (f) it had to be cut short to facilitate the preparation of the specimen for SEM examination. (g) Is a closer view of the vegetative apex inside another proliferating floret. (h, i) Show the distal end in (h) and the proximal end in (i) of a completely proliferated panicle at early stage of development. Scale bars = 1.0 mm (a–c, e, f), 15.0 mm (d) and 0.1 mm (g–i). G, glumes; L, lemma; Lp, leaf primordium; P, palea; ri, rachilla internode.

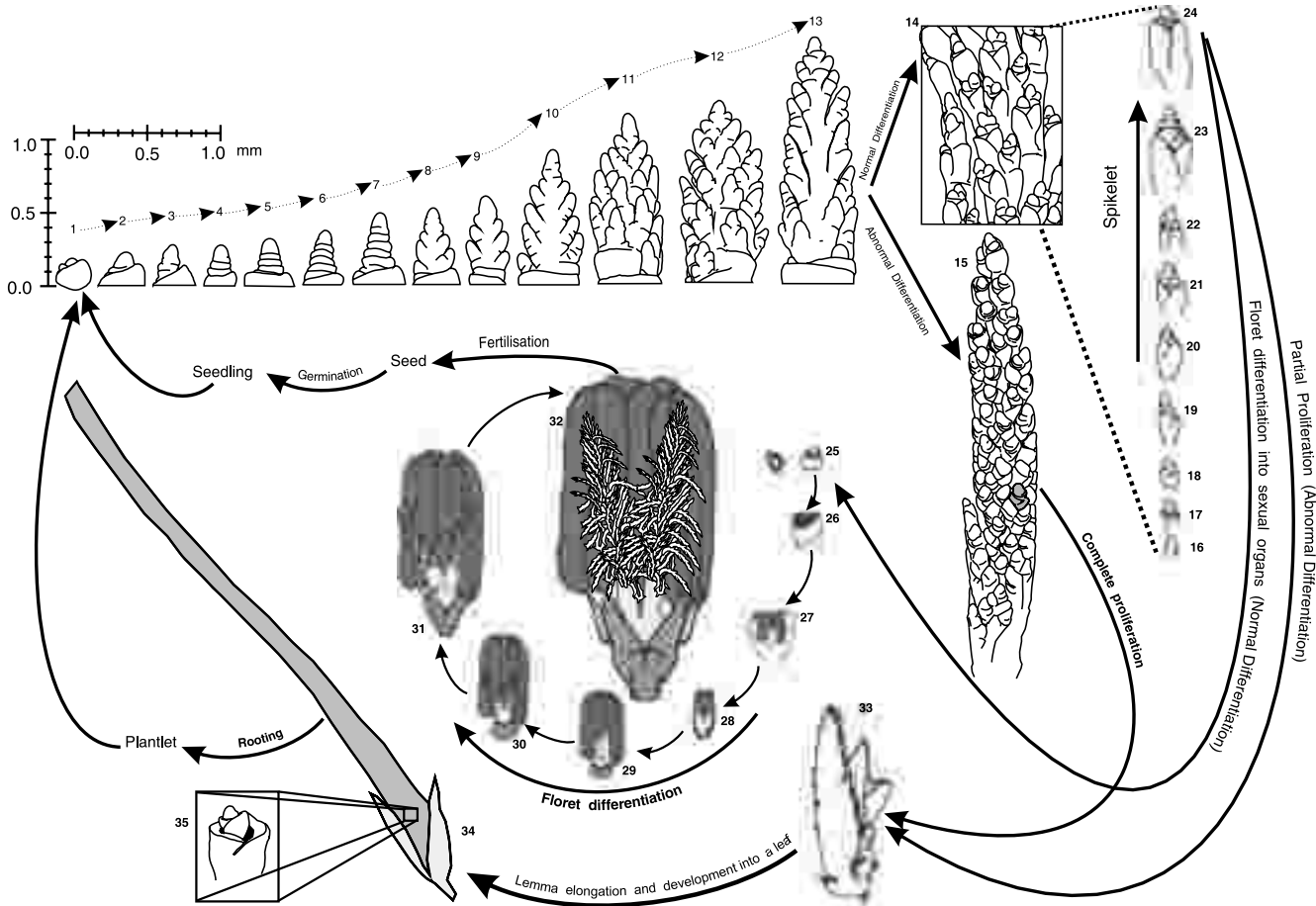


Fig. 11. An outline drawing of scanning electron micrographs covering the transition from the vegetative to the reproductive state and inflorescence proliferation in *Poa labillardieri*. Scale bar (top left) = 1.0 mm, except for no. 34, the proliferous spikelet with the elongated lemma.

a series of bracts, the lemmas, at the nodes along its length. In the axil of each lemma, a secondary axis is produced, bearing another bract (i.e. the palea), two lodicules and the sex organs.

Spikelet proliferation

From our observations on *P. labillardieri* we can place the proliferated spikelets into three categories. Spikelets in the first group have a single enlarged lemma with sexual organs in its axil (Fig. 9e, f). The pollen grains from such floral structures were viable. The second group has single or multiple enlarged lemmas developing into a foliage leaf but with no trace of sexual organs in their axils (Fig. 9g-i). The third group shows not only lemma proliferation but also prominent elongation of the rachilla segments (Fig. 10a); the proximal florets of this type have elongated lemmas with no trace of sexual organs in their axils, whereas the distal florets have both elongated lemmas and sexual organs. Since florets on the same inflorescence normally differentiate at different times and thus display a range of developmental stages, all three types of proliferations might in fact be found on the same exerted inflorescence.

SEM examination revealed no morphological difference between sexual panicles and proliferated ones in the early

stages of development before floret differentiation (Stage 5), thus agreeing with Youngner (1960) in his study on proliferation in *Poa bulbosa*.

It is evident from the above description that on the main axis of proliferated spikelets only the lemmas develop into leaves, the glumes remaining practically unaltered and the palea (when present) retaining its characters. This result was also reported by Philipson (1934) in his morphological study of the lemma in *Dactylis glomerata* and *Deschampsia caespitosa*. The leaves of the proliferated spikelets of *P. labillardieri* continue to behave as the functional equivalent of the lemmas from which they were derived because they frequently have a palea and sexual organs in their axils (Fig. 9e). In terms of development of the sexual organs in the proliferous florets, only two types were observed, including those with complete sexual organs and those without any trace of them, suggesting that there could be two distinct proliferation mechanisms rather than a single sexual suppression mechanism with varying degrees of effectiveness. There was no evidence of a positional influence on the type of expression either within spikelets or between different parts of the panicle.

Although inflorescence proliferation was noted by Vickery (1970) in some Australian species, including *P. labillardieri*, the topic has not generated much interest and there are no published

studies. For our species of special interest (*P. labillardieri*), nothing is available apart from the few lines in Vickery's paper. This neglect is unfortunate because inflorescence proliferation can be exploited as an efficient method of clonal propagation for horticultural and scientific purposes.

In a high proportion of the grasses that have shown a tendency for proliferation the chromosome numbers are extremely variable. Some of these species have a greater or lesser degree of polyploidy whereas other species have been shown to be aneuploids. In a survey on the relationship between chromosome number and the degree of proliferation in *Deschampsia alpina*, Nygren (1949) showed that plants with a diploid number of 26 were normal and produced flowers which set fertile seed, but plants with 2n numbers from 39–49 showed, with increasing chromosome number, an increasing tendency to proliferate. Thus, with increasing sterility induced by greater degrees of aneuploidy, there is a greater tendency for plants to produce proliferating spikelets.

May and Campbell (1991) in their cytogenetic and molecular study on *P. labillardieri* reported a range of ploidy levels within the species, from tetraploid to hexaploid, octoploid and dodecaploid, and noted the apparent ease with which plants of differing ploidies hybridised in the field to give pentaploids and septaploids. In terms of Nygren's suggested link between polyploidy and the tendency to proliferation (Nygren 1949), *P. labillardieri* would seem to be a good candidate for proliferous behaviour, but in the wild, such behaviour is rarely seen and limited to proliferation of only some florets within the spikelets (Vickery 1970). This may mean that there has been little selection favouring a large-scale shift in its reproductive mode from sexual to asexual (vegetative proliferation), given that pollen viability and inter-fertility is high at all ploidy levels (May and Campbell 1991; Ahmad 2005). A further factor against selection for proliferation is the extreme ease with which the species is established from seed, Campbell *et al.* (1987) noting that its capabilities in this direction have contributed significantly to its emergence as a weed in tableland pastures in New South Wales and Victoria.

In the present study, a shift was observed from occasional cases of partially proliferating spikelets that occur in the wild to complete vigorous proliferation of some panicles under greenhouse cultivation, stimulated by as yet undetermined factor(s). It is apparent that proliferation can be favoured either by the chromosome status of the species (polyploidy, aneuploidy) or by conditions unfavourable to flower production, and in most cases both of these causative factors are working together. Therefore, *P. labillardieri* is an attractive species for use in future investigations aimed at elucidating the genetical and physiological basis of this interesting phenomenon.

Acknowledgements

We warmly thank all staff of the Electron Microscopy Unit, University of Sydney, and particularly Dr Ian Kaplin and Mr Tony Romeo for their very able help in using the SEM. Thanks are also due to Professor R. A. McIntosh for assistance in the preparation of the manuscript. Finally, we thank Leppington Speedy® Seedlings and Supplies Pty Ltd for the provision of plant material and financial support.

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Manuscript received 28 April 2008, accepted 30 October 2009