Gibberellins in Immature Seeds of the Gramineae

— Chemotaxonomic considerations —

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The Gramineae is a large family and is recognized by its characteristic inflorescence. A great deal of work on the family has been done in various research ranges. In the field of biochemistry, synthesis of C_4-dicarboxylic acids as the initial products of CO_2 assimilation,[2,12] sensitivity to the herbicide, isopropyl-N-phenyl carbamate[1] and the nature of reserve carbohydrates,[6,11] have been compared within the Gramineae and discussed in relation to systematics of the family.

During the survey of endogenous GAs** in higher plants, the present author[9] observed that GA activities were detected in extracts of immature seeds of wheat, rye, and triticale by the Waito-C rice seedling assay. But no clear GA activity could be found in those of rice seeds, unless Tan-ginbozu rice seedlings were used as the assay plant.[10] Thus it was of interest to examine whether endogenous GAs of the immature seed of the Gramineae could be related to any taxonomic scheme of the family. The survey was extended to many species of the Gramineae except the Bambuseae.

Materials and methods

1) Plant materials

The ears with immature seeds were taken from 100 species of the 71 genera. They were collected in the Kanto district from 1975 to 1977 and listed in Table 1 with the date of harvesting. Fifty g fresh weight of ears with immature seeds were used for the extraction of endogenous GAs. To check changes in the quality of GAs during seed development, barley (*Hordeum vulgare* cv. Ehimehadaka No. 1) and rice plants (*Oryza sativa* cv. Ginbozu) were cultivated in the artificial field of the National Institute of Agricultural Sciences at Nishigahara in Tokyo.

2) Extraction and fractionation

Extraction, fractionation, thin-layer chromatography, and bioassay were similar to those reported in a previous paper.[7]

Sample material was homogenized in 70% aqueous acetone with a blender, kept for one day at room temperature, and filtered. After the acetone was evaporated, the aqueous solution was adjusted to pH 2.5 with phosphoric acid and partitioned three times against ethyl acetate. The combined ethyl acetate phase was extracted three times with 1 M phosphate buffer at pH 7. The buffer phase was adjusted to pH 2.5 with phosphoric acid and partitioned three times against ethyl acetate to give an acidic ethyl acetate fraction. After drying over anhydrous sodium sulfate, acidic ethyl acetate fraction was evaporated to dryness.

3) Thin-layer chromatography

The evaporated extract was taken in a small volume of acetone and subjected to TLC. Thin-layers of Silica gel H were used with the solvent system, isopropyl ether/acetic acid (95:5). The TLC plate was divided into 10 equal zones between the starting line and the

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** Abbreviations: GA(s), gibberellin(s); GAn, gibberellin An; TLC, thin-layer chromatography.

Table 1. GA activities in the extracts from ears with immature seeds of the Gramineae

Subfamily, tribe, and species	T-assay[a)]	W-assay[b)]	Date of harvesting
Pharoideae			
Oryzeae			
Leersia sayanuka	++	0	Sept. 26
Oryza sativa	++	0	Sept. 2
Arundoideae			
Arundineae			
Arundo donax var. *vesiculor*	+	0	Oct. 29
Cortaderia argentea	+	0	Oct. 3
Moliniopsis japonica	+	0	Sept. 2
Phragmites communis	+	0	Sept. 25
Arundinelleae			
Arundinella hirta	+	0	Aug. 1
Glyceceae			
Glyceria ischyroneura	++++	0	May 21
Phaenospermeae			
Diarrhena japonica	++	0	Aug. 7
Pooideae			
Agrosteae			
Agrostis clavata var. *nukabo*	+++	+++	May 14
Agrostis palustris	++	+	June 4
Alopecurus aequalis var. *amurensis*	+++	++	June 3
Anthoxanthum odoratum	++	0	May 31
Arrhenatherum elatius	+++	++	May 31
Avena fatua	++	+	May 29
Avena sativa	++	+	June 3
Beckmannia syzigachne	++	0	June 3
Calamagrostis arundinacea var. *brachytricha*	++	0	Sept. 7
Calamagrostis epigeios	+	0	June 25
Calamagrostis pseudo-phragmites	++	0	June 20
Holcus lanatus	+++	+++	June 20
Lagurus ovatus	++	++	May 26
Phalaris arundinacea	+++	+	May 29
Phalaris canariensis	+++	++	May 17
Phleum pratense	+++	+++	Aug. 24
Polypogon fugax	++	++	May 7
Trisetum bifidum	++	0	May 21
Festuceae			
Brachypodium sylvaticum	+++	++	July 22
Briza maxima	++	+	May 25
Bromus catharticus	+	0	May 29
Bromus japonicus	++	0	June 4
Bromus remotiflorus	+	0	Aug. 1
Cynosurus cristatus	++	++	June 20
Dactylis glomerata	+++	+++	Aug. 24
Festuca arundinacea	+++	+++	June 4
Festuca elatior	++++	+++	June 4
Festuca myuros	++	++	May 25

Table 1 (Continued)

Subfamily, tribe, and species	T-assay[a)]	W-assay[b)]	Date of harvesting
Lolium multiflorum	+++	+++	July 14
Lolium temulentum	+++	+++	May 19
Poa acroleuca	++++	++	Apr. 29
Poa annua	++++	++++	Oct. 26
Poa pratensis	+++	++	Apr. 21
Triticeae			
Agropyron ciliare	++	0	June 3
Agropyron tsuksiense var. *transiens*	++	0	May 29
Asperella longe-aristata	+++	++	June 20
Elymus mollis	+	+	June 5
Hordeum vulgare	++	+	May 5
Secale cereale	+++	+	May 18
Triticum aestivum	++	+	May 5
Eragrostoideae			
Chlorideae			
Chloris gayana	+	0	Sept. 12
Cleistogenes hackelii	+	0	Sept. 14
Cynodon dactylon	++	0	Aug. 9
Diplachne fusca	+++	++	Aug. 25
Eleusine coracana	+++	++	July. 26
Eleusine indica	++	+	Nov. 2
Eragrostis curvula	++	0	June 1
Eragrostis poaeoides	++	0	Aug. 13
Leptochloa chinensis	++	++	Nov. 10
Muhlenbergia curviaristata	+	0	Oct. 7
Muhlenbergia japonica	+	0	Oct. 2
Sporobolus elongatus	++	+	Sept. 15
Lappagineae			
Zoysia macrostachya	++	0	Sept. 15
Zoysia japonica	++	0	May 18
Panicoideae			
Isachneae			
Isachne globosa	+	0	Aug. 10
Paniceae			
Digitaria adscendens	++	0	July 27
Echinochloa crus-galli	++	0	July 29
Echinochloa crus-galli var. *frumentacea*	++	0	Aug. 6
Eriochloa villosa	+++	0	Aug. 4
Oplismenus undulatifolius	++	0	Sept. 7
Panicum bisulcatum	+	0	Sept. 14
Panicum maximum	+	0	Sept. 12
Panicum miliaceum	++	0	Aug. 4
Paspalum dilatatum	++	+	July 14
Paspalum distichum	+++	++	Sept. 26
Paspalum notatum	+++	+	Sept. 12
Pennisetum alopecuroides	++	0	Sept. 30
Sacciolepis indica var. *oryzetorum*	+	0	Sept. 28
Setaria chondrachne	+	0	Oct. 4
Setaria italica	++	0	Aug. 1

Table 1 (Continued)

Subfamily, tribe, and species	T-assay[a)]	W-assay[b)]	Date of harvesting
Setaria viridis	+	0	July 13
Andropogoneae			
Arthraxon hispidus	+	0	Sept. 9
Cymbopogon tortilis var. *goeringii*	+	0	Sept. 15
Eccoilopus cotulifer	++	0	Aug. 24
Hemarthria sibirica	+	0	Aug. 29
Imperata cylindrica var. *koenigii*	+++	0	Apr. 15
Ischaemum anthephoroides	++	0	Sept. 15
Microstegium japonicum	++	0	Oct. 3
Microstegium vimineum	++	0	Sep.t 26
Microstegium vimineum var. *polystachyum*	+	0	Oct. 3
Miscanthus oligostachyus	+	0	Sept. 9
Miscanthus sinensis	++	0	Sept. 30
Phacelurus latifolius	++	0	June 25
Pseudopogonatherum quadrinerve	+	0	Oct. 29
Pseudopogonatherum speciosum	+	0	Nov. 8
Sorgum bicolor	+	0	Aug. 4
Sorgum halepense	++	0	July 30
Spodiopogon sibiricus	++	0	Aug. 24
Themeda japonica	+	0	Sept. 15
Maydeae			
Coix lacryma-jobi	++	0	Aug. 9
Zea mays	+++	0	Aug. 19

a) Tan-ginbozu rice seeding assay, C-3-hydroxy and C-3-deoxy GAs are detectable.
b) Waito-C rice seedling assay, C-3-hydroxy GAs are detectable but not C-3-deoxy GAs. Relative GA activities: ++++ very high, +++ high, ++ moderate, + low, 0 very low to inactive.

solvent front (the first zone was further subdivided into two zones). The scraped Silica gel of each zone was eluted with 50% acetone, evaporated to dryness, and dissolved again in 0.1 m*l* of 50% acetone for bioassay.

4) Bioassay

Two dwarf rice, Tan-ginbozu and Waito-C, were used to assay GAs. Tan-ginbozu is known to respond to many kinds of GAs, while Waito-C responds to limited kinds such as C-3-hydroxy GAs.[8)] Such specificity between the two dwarfs was used to identify the GAs found in the extracts.

When the second leaf emerged from the first leaf, eluates were applied as a single 1 μl droplet to the surface of each coleoptile with a micropipette. Test plants were grown at 32°C under continuous light of about 5000 lux. After 3 days the length of the second leaf sheath was measured with a ruler. The result was summarized as histograms, each indicating the average of 5 test plants.

Results and discussion

In the beginning, it was examined whether endogenous GAs of a species of the Gramineae are always active or inactive to the elongation of Waito-C seedlings during its seed development. Forty ears of barley and those of rice were harvested at their several growth stages after anthesis for the extraction of GAs.

Fig. 1 shows results of barley ears. GA activities detected by the Tan-ginbozu assay

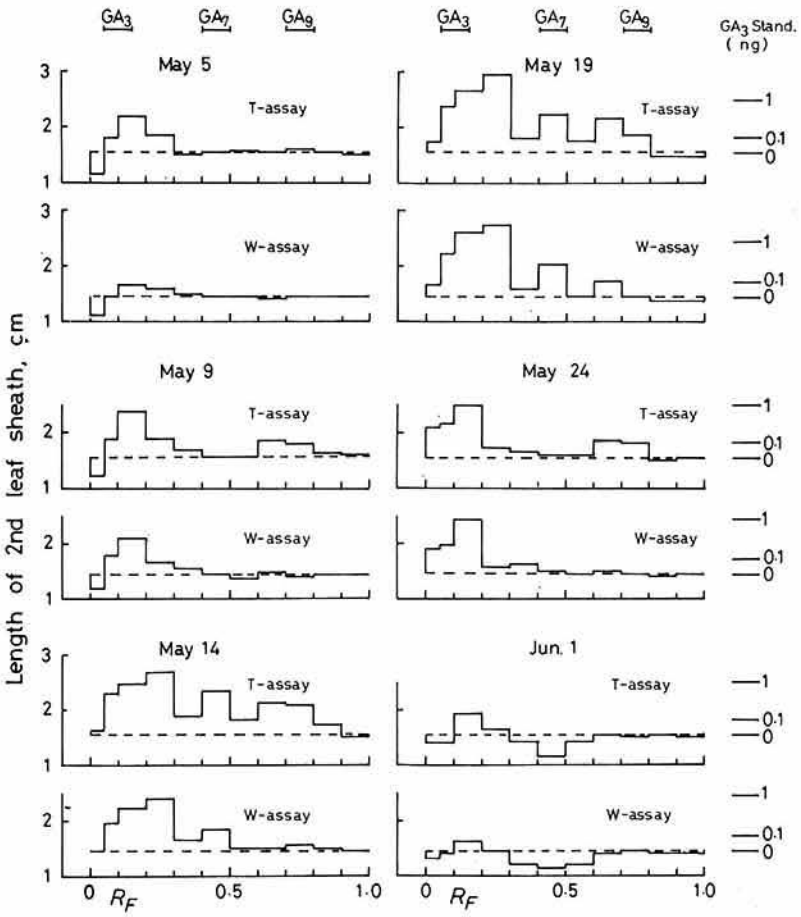

Fig. 1. Histograms showing GA activities of extracts taken from barley ears at different stages of ripening

The acidic ethyl acetate fractions were separated with TLC using isopropyl ether/acetic acid (95 : 5) as the developing solvent. Eleven eluates were tested on Tan-ginbozu rice seedlings (T-assay) or Waito-C rice seedlings (W-assay). This legend also applies to Fig. 2–Fig. 7.

were low in the ears of May 5, 7 days after anthesis, and those of June 1 at the mature stage. However, GAs were detected by Waito-C seedlings as well. Fig. 2 shows results of rice ears. At any ripening stage GA activities could not be found in the histograms when Waito-C seedlings were used as the assay plant, while clear GA activity was detected by Tan-ginbozu rice seedlings. This activity is due to GA_{19} as already reported.[5,10] Thus it is a characteristic nature independent of its ripening stages whether the ear extract is active or inactive on the Waito-C assay.

The occurrence of endogenous GAs in the extracts from ears with immature seeds are summarized in Table 1. The system used by Tateoka[13] is employed here for classification of the Gramineae. The table was reproduced on the basis of the histograms showing GA activities, some of which are shown in Fig. 3 to Fig. 7.

The extracts of 2 species of the subfamily

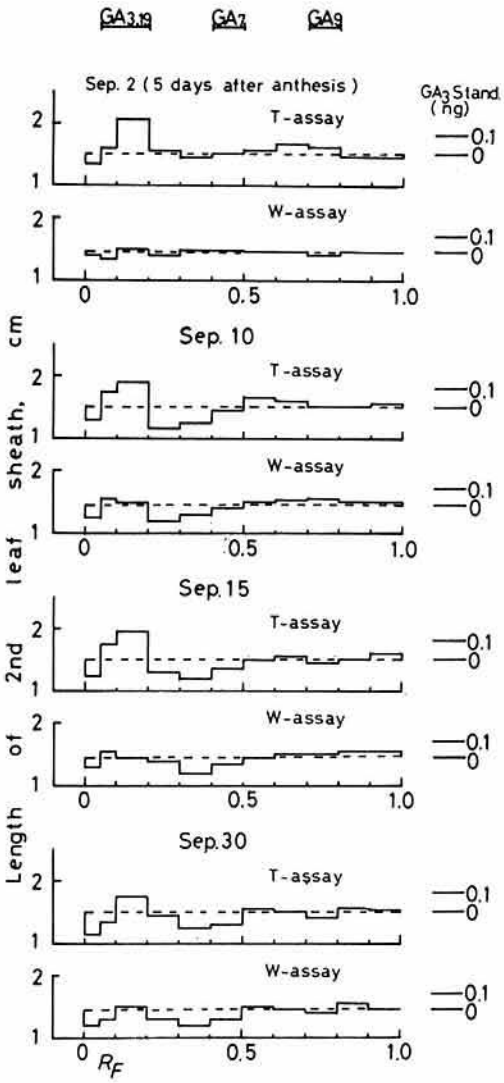

Fig. 2. Histograms showing GA activities of extracts taken from rice ears at different stages of ripening

Pharoideae and those of 7 species of the subfamily Arundoideae were all inactive on the Waito-C seedling assay.

With respect to the subfamily Pooideae, containing the tribes Agrosteae, Festuceae and Triticeae, Waito-C seedlings were not responsive to extracts of 11 species belonging to 6 genera. In other 29 species out of 21 genera, GA activities were detected with both dwarf rice seedlings. The histograms of 6 species of the Agrosteae and those of the Festuceae are shown in Figs. 3 and 4, respectively. Every species in these figures has a clear and high GA activities by both rice seedlings. This means that these species contain higher concentrations of C-3-hydroxy GAs such as GA_3 in immature seeds. As shown in Table 1, no C-3-hydroxy GA was detected in 3 species of *Calamagrostis*, 3 species of *Bromus*, and 2 species of *Agropyron*. All species surveyed in the genera *Festuca*, *Lolium* and *Poa* contained C-3-hydroxy GAs. Jones et al.[4] have reported that GA_3 was identified in extracts of immature seeds of *Lolium perenne*, *Dactylis glomerata*, and *Festuca pratensis* by techniques based on TLC, fluorimetry and bioassay on peas. The histograms of Fig. 4 clearly indicate the presence of GA_3 in immature seeds of *Lolium multiflorum*, *Dactylis glomerata*, and *Festuca arundinacea*. There may be some relationships between a taxonomic group and the occurrence of a particular GA in immature seeds of the Gramineae.

The extracts from 5 species of the subfamily Eragrostoideae, *Diplachne fusca*, *Eleusine corcana*, *Eleusine indica*, *Leptochloa chinensis*, and *Sporobolus elongatus* had GA activities on both Tan-ginbozu and Waito-C rice seedlings. Extracts from other 9 species were inactive on the Waito-C assay. Representative histograms of the tribe Chlorideae of the Eragrostoideae are presented in Fig. 5. According to the manual of Hitchcock,[3] the group of the Chlorideae is heterogeneous. The genera *Leptochlora*, *Eleusine* and *Diplachne* are more nearly related to certain genera of Festuceae. In the present research, 3-hydroxy GAs, which are active on Waito-C seedlings and characteristic of the Festuceae, were detected in the endogenous GAs of these species.

Within the subfamily Panicoideae, containing the tribes, Isachneae, Paniceae, Andropogoneae, and Maydeae, no clear GA activity was detected except for 3 species of the genus *Paspalum* of Paniceae, when Waito-C rice seedlings were used as the test plant. The histograms of 6 species of the Paniceae and

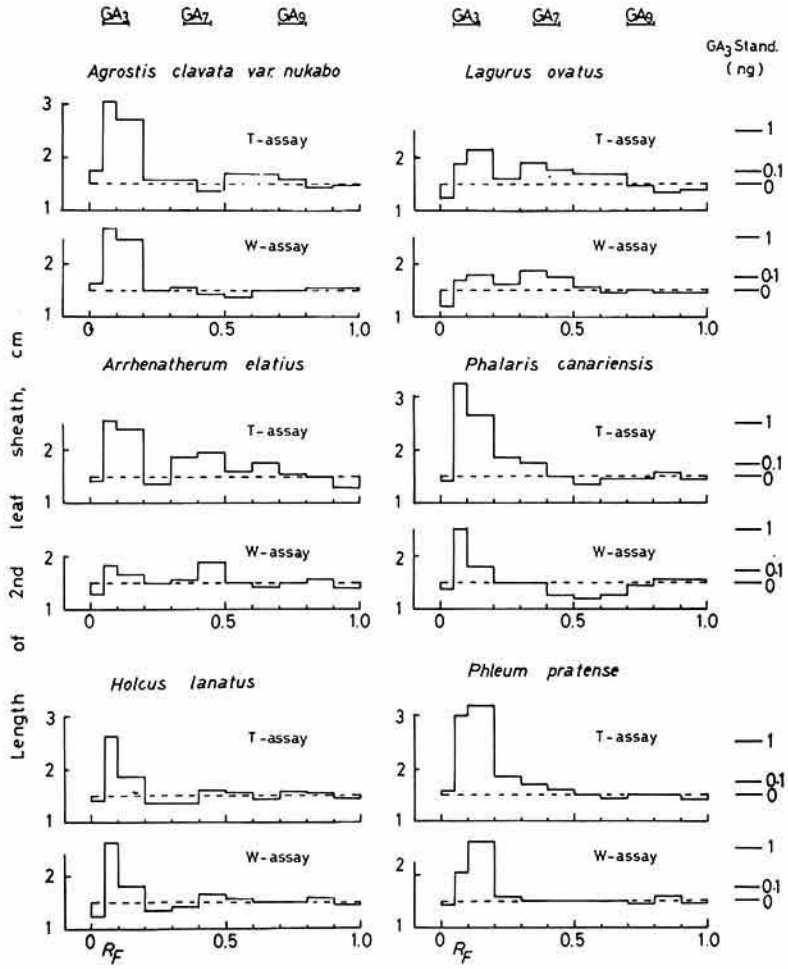

Fig. 3. Histograms showing GA activities of extracts taken from ears with immature seeds of the tribe Agrosteae

those of the Andropogoneae are shown in Fig. 6 and Fig. 7, respectively. The histograms of the Andropogoneae in Fig. 7 show that the GA activity appeared at the R_F value corresponding to GA_{19}.

The Gramineae is subdivided into two contrasting subfamilies, the Festucoideae (Pooideae) and Panicoideae on the basis of inflorescence characteristics.[3] In this taxonomic system, the Panicoideae contains the tribes, Arundinelleae, Isachneae, Paniceae, Andropogoneae, and Maydeae, defined by Tateoka. In the Panicoideae of this system, only 3 species of the genus *Paspalum*, *P. dilatalum*, *P. distichum*, and *P. notatam*, contain C-3-hydroxy GAs, which are active on both dwarf rice seedlings. From geographical distribution, the Panicoideae is a group of grasses usually found in the tropical and warm regions. Non-C-3-hydroxylated GA such as GA_{19} is the major GA of this group. The Pooideae defined by Tateoka is a group of grasses in cool and temperate regions. Most of the members of this group contain C-3-hydroxy GA which is active in both Tan-ginbozu and Waito-C seedlings. The present study indicates that there

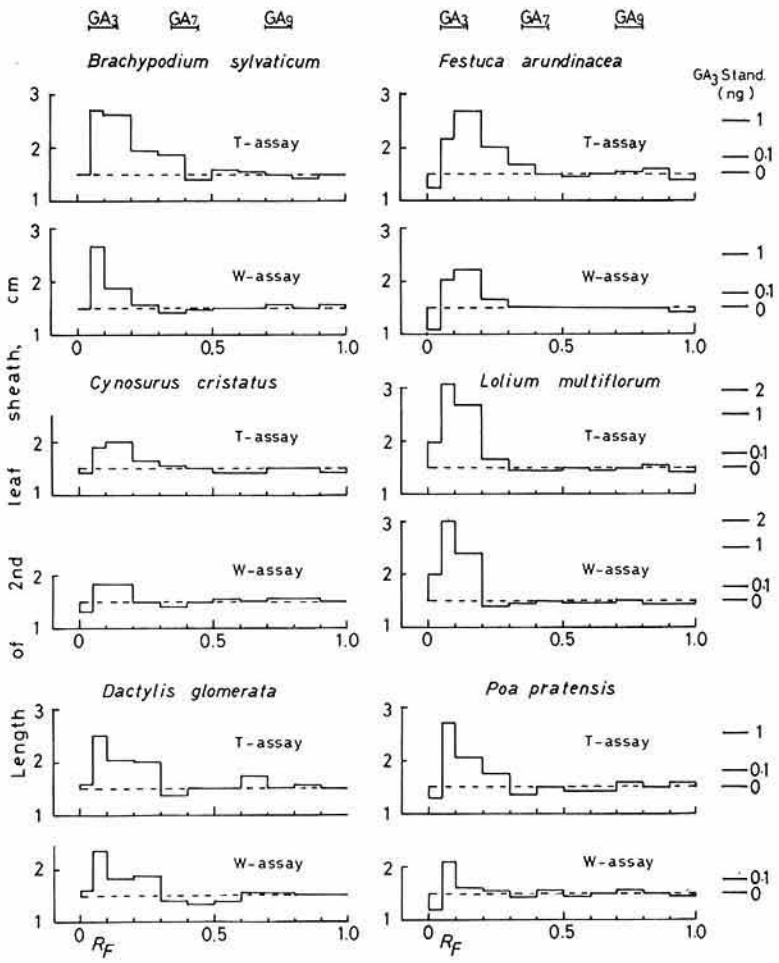

Fig. 4. Histograms showing GA activities of extracts taken from ears with immature seeds of the tribe Festuceae

is a difference of metabolic pathway of GA between the Pooideae and the Panicoideae.

Conclusion

Endogenous GAs in immature seeds of 100 species belonging to 71 genera of the Gramineae were studied by techniques based on TLC and bioassay on dwarf rice. GAs were detected in all species by using Tan-ginbozu rice seedlings. When another dwarf variety, Waito-C was used, GAs were detected in 37 species belonging to 26 genera. This is due to the fact that Tan-ginbozu seedlings respond to many kinds of GAs, while Waito-C seedlings respond to limited kinds such as C-3-hydroxy GAs. In conclusion, most of the members of the Pooideae contain GAs hydroxylated at C-3 in immature seeds. However major GAs of immature seeds of the Panicoideae are not hydroxylated at C-3.

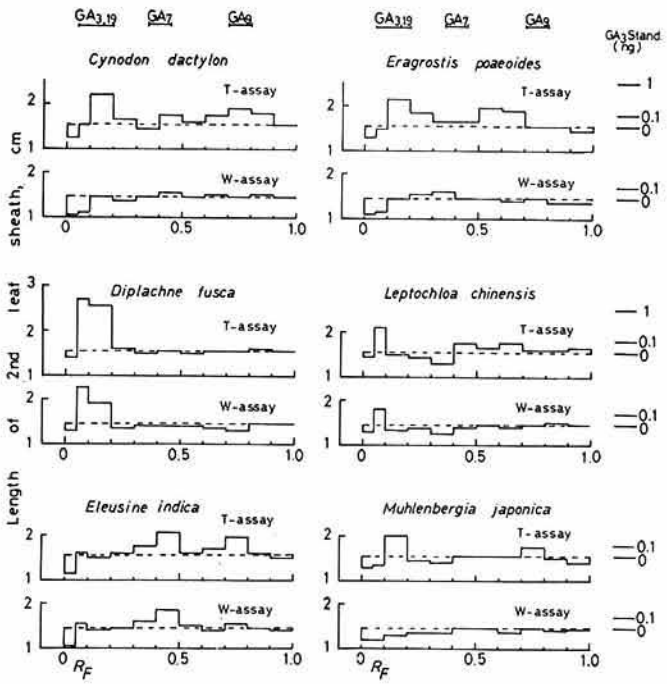

Fig. 5. Histograms showing GA activities of extracts taken from ears with immature seeds of the tribe Chlorideae

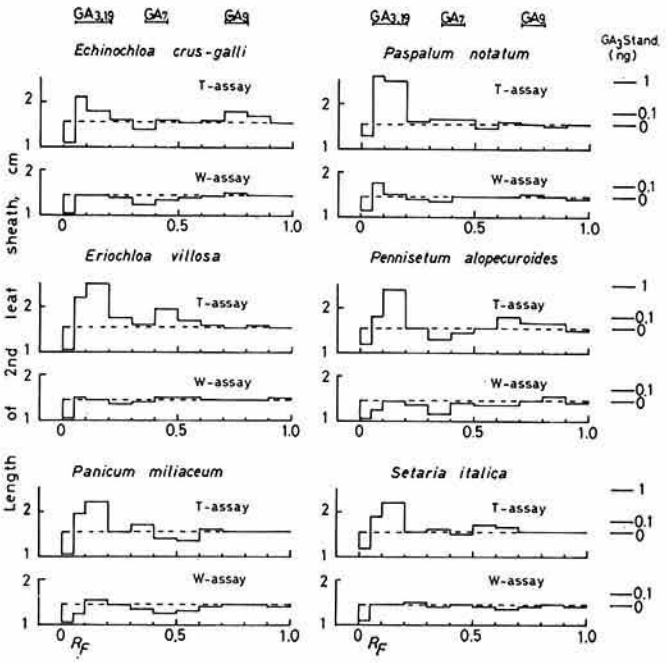

Fig. 6. Histograms showing GA activities of extracts taken from ears with immature seeds of the tribe Paniceae

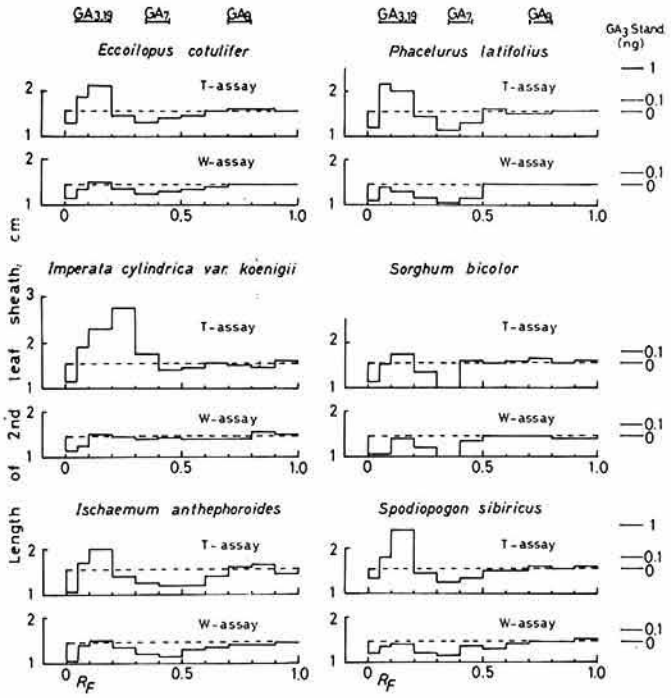

Fig. 7. Histograms showing GA activities of extracts taken from ears with immature seeds of the tribe Andropogoneae

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