CHAPTER 6

DISCUSSION AND CONCLUSION

6.1 Specimen collection

In this chapter, plants names are referred to according to Irwin and Barneby (1981). In Flora of Thailand 23 taxa of the genus *Cassia* L. were enumerated (Larsen et. al.,1984). Many attempts have been made to collect all the species throughout the country (Figure 4.1). However, 18 out of 23 taxa were collected and used in this study (Table 5.1). The 5 missing taxa are *Cassia javanica* subsp. *nodosa* (Buch.-Ham. Ex Roxb.) K & S.S. Larsen, *C. agnes* (de Wit) Brenan, *C. fruticosa* Mill, *C. mimosoides* L., *C. bicapsularis* L. and *C. absus* L.. It should be noted that *Cassia fruticosa*, *C. bicapsularis* were not common and *Cassia absus* was only once collected from Khao Tao at Hua hin (Larsen et. al., 1984).

In addition to the 23 taxa mentioned in the Flora of Thailand, *Cassia obtusifolia* L., was found in a small population during specimen collection in Thong Pha Phum District, Kanchanaburi Province. From the description of species in 5.2, *Senna tora* and *S. obtusifolia* are very similar, but they can be distinguished in number of gland on rachis. It was found that *S. tora* has one gland and *S. obtusifolia* has two. *Senna obtusifolia* was reported in Flora of Malesiana (Larsen and Hou, 1996). In this respect, *Cassia obtusifolia* is a new recorded species for Thailand.

6.2 Numerical taxonomy

The numerical taxonomy can use to classify many living organism, for examples, ferns, flowering plants in any taxon such as family, genus, species and variety (Sneath and Sokal, 1987), such as *Pyrrosia* (Polypodiaceae), *Poa* (Poaceae), *Solidago* (Asterceae) so on and so forth (Rossarin, 1996; Semple, Chmielewski and Brammall, 1990; Giussani, Martinez and Collantes, 1996). In

this study, quantitative characters of *Cassia* s. l. were used to analyze in three multivariate techniques:- factor analysis, cluster analysis and discriminant analysis.

6.2.1 Factor analysis

PCA is a useful tool for categorization of data, since it separates the dominating features in the data set. The eigenvalues for each principal component corresponds to the amount of total variance in the data described by this component (øieroset, 1999). Thirty-two quantitative characters were used in this analysis. It is found that 7 factor components have eigenvalue more than 1(Table 5.2). It shows that these 7 factor components are representatives of all variables or characters (see Supot Saengmanee, 1999: 173). In addition, factor loading (Table 5.3) and communality values (Table 5.2) of all variables are more than 0.5, these meant that variable in each factor have some relationships and suitable to use to explain the variance of the population (Supot Saengmanee, 1999: 173). It was found that 85.5% of the variance could be explained by 7 factors. The variables which contribute to the segregation along the first principal component are related to leaf size, i.e. RCD, TLL, TWL, LMW, DBLP, BTWP, POLL, PET, and NOL and along the second principal component are related to flower size, viz. PCL, FLD, PTL, FTL, OVL, FML, OSL, PTW, and STD. While the third principal component are related to the remainder of leaf and flower (size), i.e. BTW, BTL, PED, RCL, AND, and ANL. This result is in agreement with the multivariate analysis of variation in *Ranunculus decurvus* (Hook.f) Melville and *Ranunculus concinnus* (Hook.f) Melville (Menadue and Crowden, 1988). They concluded that there is no resolution into groups when all the specimens are considered.

6.2.2 Cluster analysis

The dendrogram split the 508 specimens into either two and four groups (Figure 5.4) at the 1.80 or 1.30 levels, respectively. In the two-cluster groupings, specimens classified as group 1 consisted of all members from *Chamaecrista pumila* and *Chamaecrista leschanaultiana*. Specimens classified as group 2 formed three subgroups. Subgroup 1 consisted of all member from *Senna*

alata, subgroup 2 included all but S. alata and S. spectabilis. Subgroup 3 comprised *Senna spectabilis* and 4 species of *Cassia* s.s., *viz. C. fistula*, *C. javanica* var. *javanica*, *C. grandis*, *C. bakeriana*. In the four-cluster grouping, group 1 was the same as in the two cluster grouping and consisted of all members of the genus *Chamaecrista*. All member of *S. alata* were placed in group 2, group 3 included members from the 10 species of *Senna*, and excluding *S. alata* and *S. spectabilis*, and group 4 consisted all the Cassia s.s., and *Senna spectabilis*.

Both the two and four-cluster groups demonstrated a separation of the genus *Chamaecrista*, the clear cut separation of this genus from the remainder is probably due to their extreme difference in leaf and flower characters from the other. Whereas there are close relationships on the dendrogram among the *Senna* and *Cassia* s.s. The separation of *Senna alata* from its original group is probably caused by its largest leaf size among the *Cassia* s. l. in Thailand, though its flower characters are similar with other *Senna*.

In the case of *S. spectabilis*, this species is a medium-sized tree like all members of the genus *Cassia* s.s. that it was included in to the *Cassia* s.s. revealed a closer relationship to *Cassia* than *Senna*. Table 5.1 shows 18 taxa used in this study, it can be seen that the genus *Senna* has two habits, i.e. shrub and tree while the entire genus *Cassia* s.s. are trees. It seems likely that the tree members of the genus *Senna* and the genus *Cassia* s.s. are rather close. Such relationship is likely related to flower and leaf characters of *S. spectabilis* which are similar to the overall *Cassia* s.s. Within group 4, *S. spectabilis* shows a closer relationship with *C. javanica* var. *javanica*, *C. grandis*, *C. bakeriana* than *C. fistula*. The number of leaflets of *C. fistula* is less than *S. spectabilis* and leaf size of *C. fistula* are larger than *S. spectabilis* (Table 5.24). These leaf features may be in part made the two species slightly separated (Figure 6.1).

The result of four-cluster groups may be comparable to the results on stem, leaf and flower anatomy of the *Cassia* s. l. in Thailand by Kidyu (2001). He concluded that the *Cassia* s. l. should be divided into 4 groups. However, in his work the genus *Cassia* s.s. is not included *S. spectabilis*. Moreover, *C. alata* was still included in the group *Senna*.

6.2.3 Discriminant analysis

In this analysis, four criteria were used. In the first criterion, the 18 taxa were divided into 18 categories according to Larsen et al. (1984). The second criterion using a priori results from the cluster analysis, i.e. 4 categories. The third criterion, the 18 taxa were divided into 4 categories according to the result of stem, leaf and flower anatomy of the *Cassia* s.l. (Kidyue, 2001). Finally, the 18 taxa were divided into 3 categories according to Irwin and Barneby (1981).

From overall canonical discriminant analyses, it can be seen that the ordination plot on the two canonical axes (Fig 5.7a, Fig 5.8a, Fig 5.9a, Fig 5.10a, Fig 5.11a and Fig 5.12) shows that the 18 taxa of the *Cassia* s.l. are separated into 3-4 groups. The most important variable to separate the three groups on axis 1 is the filament length (FML) whereas lamina width (TLW), anther length (ATL), petiolule length (POLL) and petiole diameter (PED) are important character on axis 2. It can be concluded that the classification of the *Cassia* s.l. into 18 taxa of a single genus, *Cassia* L. as mentioned in the Flora of Thailand (Larsen et al., 1984) was not appropriate according to the results of canonical discriminant analyses from this study.

As the 4 categories of the canonical discriminant analysis are followed the result of cluster analysis. The ordination plot on the two canonical axes (Figure 5.10) shows that the four categories are not distinct. It was found that the four-cluster grouping according to the result of cluster analysis is not relevant with the result from canonical discriminant analysis.

When the 3 categories canonical discriminant analysis were conducted following the result of stem, leaf, and flower anatomy of the *Cassia* s.l. (Kidyue, 2001). He separated the *Senna* into Senna-1 and Senna-2 according to habit (tree or shrub) and the stomatal distribution on leaves (hypostomatic or amphistomatic leaf). It can be concluded that 18 taxa of the *Cassia* s.l. were separated into three groups on both of canonical axis 1 and canonical axis 2. It was found that clusters of *Senna*-1 pooled with cluster of *Senna*-2 in both canonical axes. The result from this study indicated that *Cassia* s. l. in Thailand should divide into three groups,

and the 4 grouping as was mentioned from the results of qualitative plant anatomy are still not pertinent.

As was mentioned in chapter 3, some workers recognized the three segregated genera, viz. *Cassia* s. s., *Senna* and *Chamaecrista* from the *Cassia* s. l., using characters of stamen, bracteole, seed coat, etc. (Irwin & Barneby, 1981; Lock, 1988; Mabberley, 1997). While using the 3 categories as a priori groups according to Irwin and Barneby (1981). The ordination plots of the canonical discriminant analysis (Figure 5.12) show that 18 taxa of the *Cassia* s. l. in Thailand was divided into three groups. All of the most highly associated characters with canonical axis 1 are characters of flower.

In all, there is justification to indicate the presence of three distinct genera in the *Cassia* s. l. based on the result of canonical discriminant analyses. Some recently works on this plant group also supported this numerical study. For example, Tucker (1996) investigated and compared features of inflorescence and floral organ initiation and development among one species of *Cassia* s. s., six species of *Senna* and two species of *Chamaecrista*, he concluded that distinction in floral ontogeny supported the segregation of these three genera. Recently, Ghareeb, Khalifa and Fawzi (1999) working on electrophoretic seed protein, chromosome number and morphological characters of 10 species of the genus *Cassia* s.l. belonging to subgenera *Fistula* and *Senna*. The results obtained from their work support the earlier taxonomic treatments of the genus *Cassia*.

6.3 Comparision of qualitative morphological characters of the *Cassia* s. l.

The *Cassia* s. l. have some common morphological characters. For example, they have uni-pinnately compound leaves, 5 petals and 5 sepals, 10 stamens (3 large, 4 smaller, and 3 reduced) and superior ovary with ovary stalk from the result of comparative morphological study of the 18 taxa (Table 5.26).

If we considered plant habit in the *Cassia* s. l. from the 18 taxa. There are three kinds of habit, viz. tree, shrub and herb. *Cassia* has only one habit, i.e. tree and *Chameacrista* is quite a small shrub while *Senna* owns both of tree and shrub.

C. grandis has three recurved filaments and small flowers. The diameter of flower and their leaflet size is comparable to *Senna*. Whereas morphological character of *C. javanica* and *C. bakeriana* are very close, these two species are frequently misidentified. However, the pubescence of leaf, ovary and fruit in *C. bakeriana* and the bigger size of flower diameter can distinguish them. *Senna spectabilis* has straight filament while flower diameter is close to *Cassia* s.s. due to it have spatulate petal. Moreover, *S. alata* obtains large leaflets which is nearly the same size as *C. fistula*.

In this study there are two pair of closely related taxa. The first pair is *Cassia surattensis* subsp. *surattensis* and *Cassia surattensis* subsp. *glauca*, all characters of these two species are comparable but they are differed in size which the first species smaller than the latter. The second pair is *S. tora* and *S. obtusifolia* are also very similar, but they can be distinguished in number of gland on rachis. It was found that *S. tora* has one gland and *S. obtusifolia* has two.

6.4 Numerical Taxonomy and closely related taxa

In section 6.3 two pairs of closely related taxa were noted. It is interesting to investigate how numerical taxonomy can recognize these related taxa. Figure 5.4 shows the result of UPGMA clustering of 508 OTUs based on 32 characters of the *Cassia* s.l. in Thailand. It can be seen that the condensed dendrogram could not demonstrate detailed position of each specimen. However, from the expanded dendrogram, Figure 5.4 can be slightly added some more details of the positions of (1) *Cassia surattensis* subsp. *surattensis*, and *Cassia surattensis* subsp. *glauca* (*Senna glauca*), and (2) *Senna tora* and *Senna obtusifolia* (Figure 6.1). Figure 6.1 shows specimens of *Cassia surattensis* subsp. *surattensis* and *Cassia surattensis* subsp. *glauca* are grouped in the *Senna* group, but in rather far apart clusters. It is indicated that the two taxa are distinct, probably at the level of species. However, this finding is only the result from quantitative characters. In contrast, *Cassia surattensis* subsp. *surattensis* and *Cassia surattensis* subsp. *glauca* are placed in the same species without infraspecific taxa (Larsen and Larsen, 1980; Hou, Larsen and Larsen, 1996). Hou, Larsen and Larsen (1996) also treated *Cassia surattensis*

subsp. *surattensis* as *Senna surattensis* (Burm.f.) Irwin & Barneby without infraspecific taxa, but they did not mentioned *Cassia surattensis* subsp. *glauca*.

Figure 6.2 shows the ordination plot of 12 species of *Senna*. It can be seen that *Cassia* (*Senna*) *surattensis* subsp. *surattensis* (12) and *C.* (*Senna*) *suattensis* subsp. *glauca* (11) are not distinct. This result is in agreement with the treament of *Senna surattensis* without infraspecific taxa by Hou, Larsen and Larsen (1996) in Flora Malesiana.

In the case of *Senna tora* and *Senna obtusifolia* the dendrogram (Figure 6.1) shows a close relationship of this two species. As their undershrub habit (Table 5.1), these two species are in separate cluster with the other *Senna* species. This cluster grouping agrees well with the presently known species in the Flora Malesiana (Hou, Larsen and Larsen, 1996). From the result of canonical discriminant analysis, *Senna obtusifolia* (7) and *S. tora* (14) are slightly distinct. This result confirms a close relation of the two species.

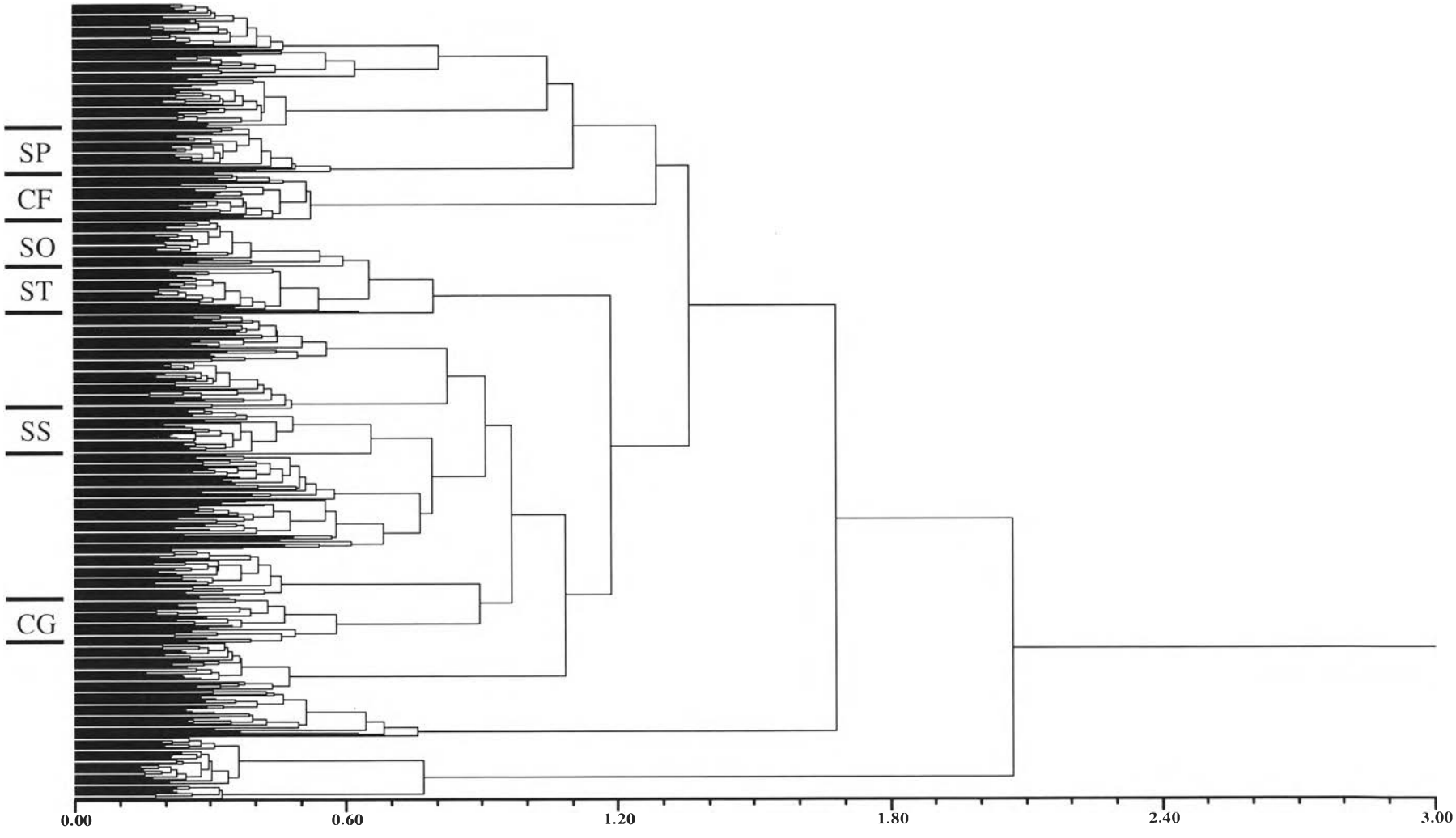


Figure 6.1 UPGMA clustering of 508 OTUs based on 32 Characters of *Cassia* s.l. in Thailand (SP- *C.(Senna) spectabilis*, CF-*C. fistula*, SO- *C.(Senna) obutusifolia*, ST- *C.(Senna) tora*, SS- *C.(Senna) surattensis* subsp. *surattensis*, CG-C. *surattensis* subsp. *gluca*)

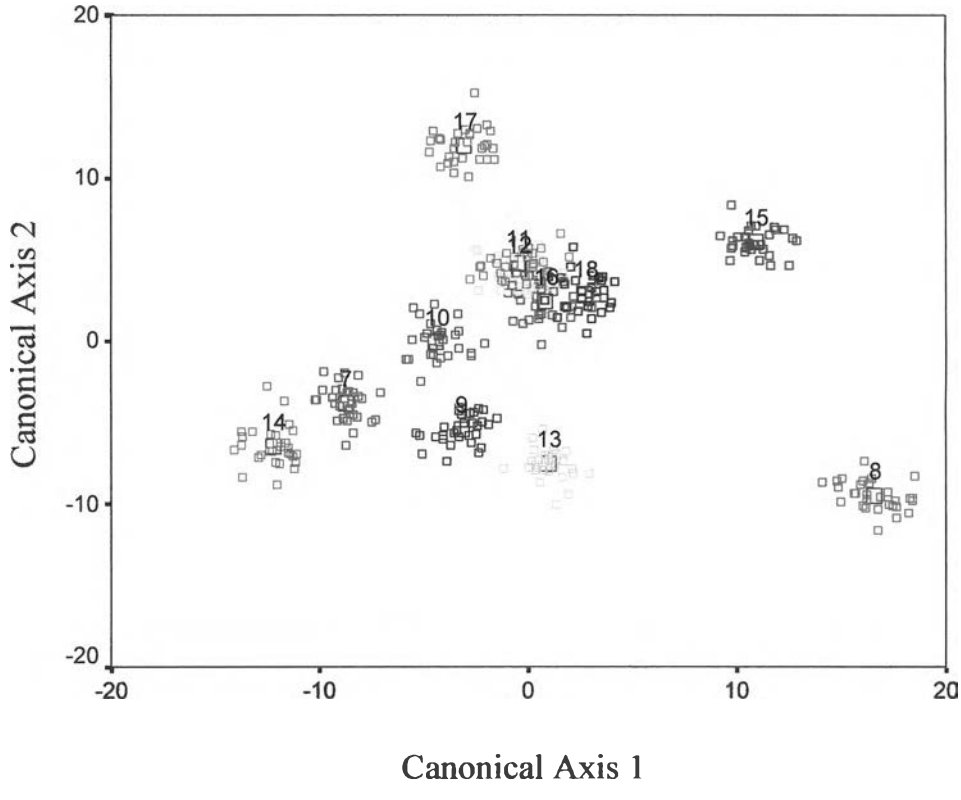


Figure 6.2 The ordination plot of 12 species of *Senna.*

6.5 Conclusion

Three techniques of numerical taxonomy were used to investigate the taxonomic status of 18 taxa in the *Cassia* s. l. in Thailand. It should be concluded that the results from numerical taxonomic study as well as the comparision of qualitative characters of the *Cassia* s. l. provide justification for recognition of the segregation of the three genera, namely *Cassia* s. s., *Chameacrista* and *Senna* from the *Cassia* s. l. This result is in agreement with Irwin and Barneby (1981). However, it should be noted that *S. alata* is rather unique in their leaf size and somewhat separated from its original group. The introduced species for road tree, *S. spectabilis* occurs naturally in tropical America. This species is rather close to the *Cassia* s.s., especially *C. fistula* from the results of cluster analysis. This present finding reveals some heterogeneities within the genus *Senna*. In addition, the treatment of the genus *Senna* as a genus retains at least three subgenera seems to be possible.

From the ordination plot of 12 species of *Senna* (Figure 6.2), the *Senna* can be divideed into 3 subgroups or subgenera. Subgroup 1 consisted of *S. alata*, subgroup 2 comprised *S. timoriensis*, and subgroup 3 included the remainder species. Because the distinction between *S. alata* and the remainder species was observed on canonical axis 1, which is 99.1% correlated with all the characters, and the variance explained by it is 31.0% (Table 6.1). However, the result of canonical discriminant analysis did not support the separation of *S. spectabilis* from the remainder.

Box plots of ten most important characters are demonstrated in Figure 6.3. It can be seen that filament length, fruit length and ovary stalk length are useful quantitative chartacters for discriminating the three genera. The following is an identification key to the genera of the former *Cassia* L. in Thailand.

1a	The longest filament recurved, more than 2.5 cm long.....	**1.** ***Cassia***
1b	The longest filament straight, less than 1 cm long.....	2
	2a Ovary stalk more than 0.9 mm long, fruit more than 9 cm long.....	**2.** ***Senna***
	2b Ovary stalk less than 0.9 mm long, fruit less than 9 cm long.....	**3.** ***Chamaecrista***

In all, numerical taxonomy can reexamine the principles of taxonomy and of the proposes classification. This has benefited taxonomy in general, and has to lead to the posing of some taxonomic questions as was noted by Sneath and Sokal, (1973).

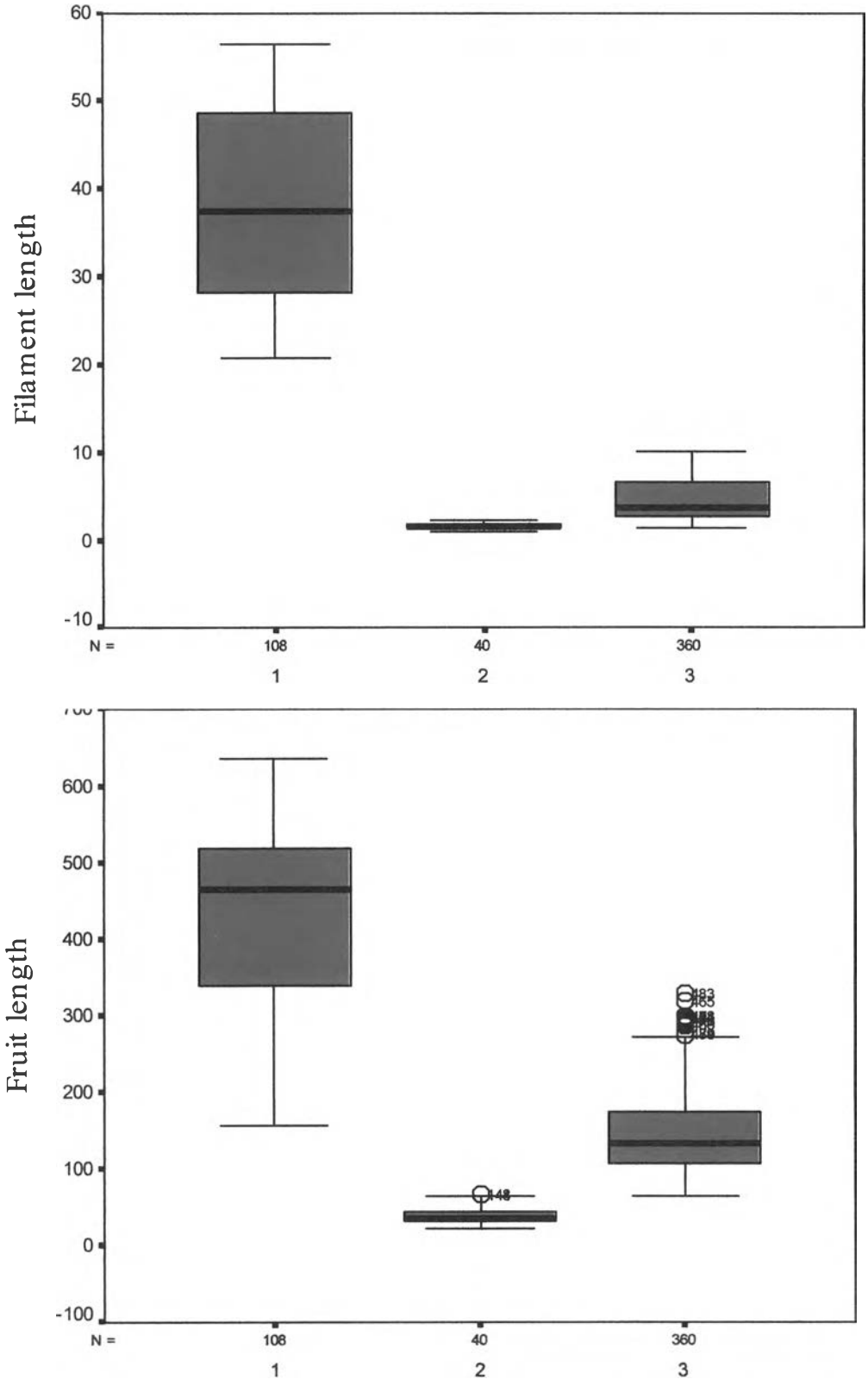
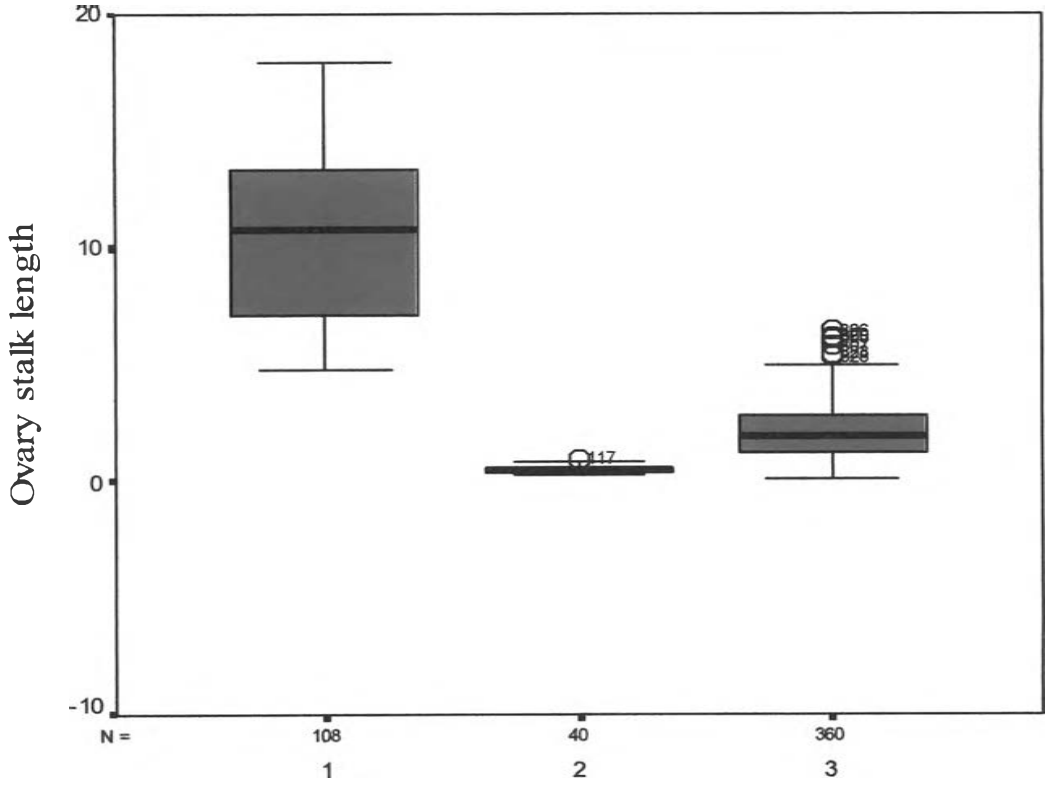


Figure 6.3 Boxplots of the ten more important characters of *Cassia* s.l. (1-*Cassia* s.s., 2-*Senna*, 3-*Chamaecrista*)



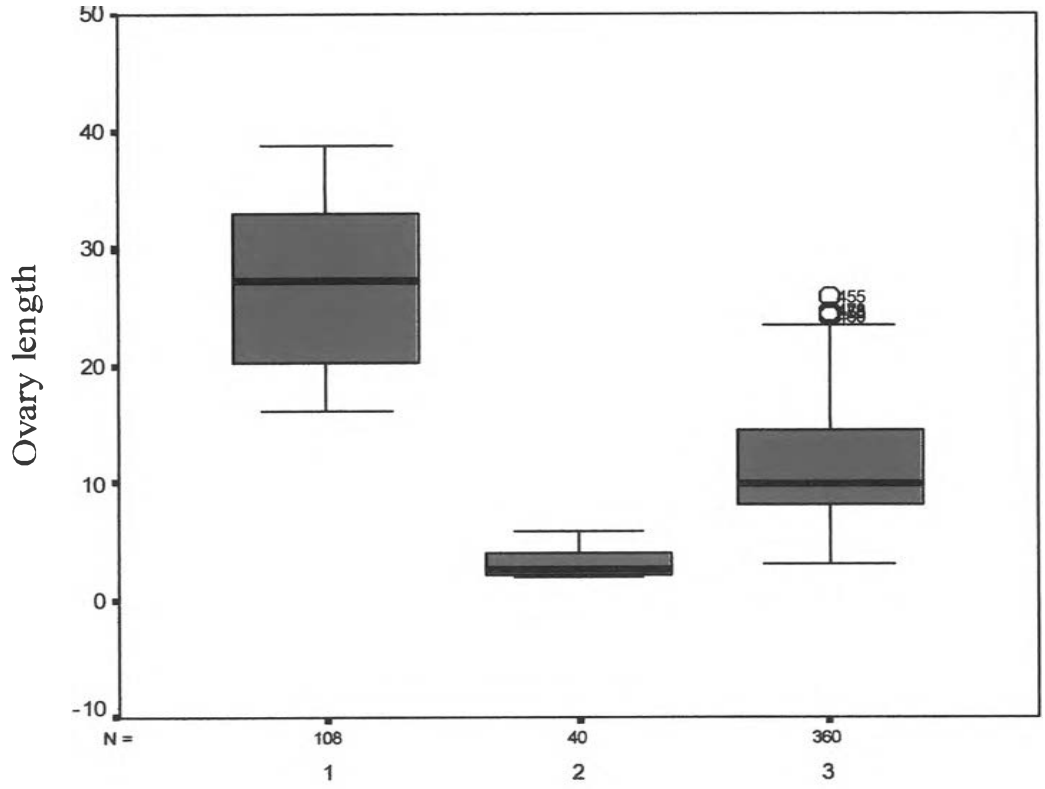


Figure 6.3 (continued)