ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

# Species Distinctiveness in *Trifolium quartinianum* A. Rich and *T. steudneri* Schweinf. (*Leguminosae*)

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Addis Ababa

July 2004

# ACKNOWLEDGMENTS

I would like to acknowledge my advisors, Drs. Jean Hanson, Kifle Dagne, and Ensermu Kelbesa. I would also like to acknowledge the Department of Biology and the Graduate School. The International Livestock Research Institute (ILRI) is also acknowledged. Professor Legesse Negash is also given much credit for generously allowing me use the laboratory that is under his supervision.

Mekelle University deserves, and is given, much credit for sponsoring me through the graduate study. Ato Tadesse Dejene, Ato Tsehaye Asmelash, and all members of Mekelle University are best appreciated. W/ro Weinshet Assefa and W/ro Milete Berhe and Ato Anteneh , from AAU are also appreciated for their help.

I owe a lot to my father, the first teacher of mine, for his golden words and deeds. My mom is given the best credit ever for all the unutterable love and directions she gave me; god bless you, mom. I would also like to give whole- hearted acknowledgment and immense love to the strong and beloved wife of mine, W/ro Yigerem Girmay (Lily), and adored baby Fikir Melkamu, to whom I dedicate this work.

Last but not least, I would like to acknowledge my friends, Ato Mekonnen Tsegaye, Ato Abebe Amha, Ato Solomon Teka, Ato Dagnachew Worku, Ato Mohammed Ahmed, Adey Feleke and a lot more precious ones for being there in all times with me; thank you so much. All friends and persons who helped me directly or indirectly are unforgettable forever.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# ABSTRACT

Morphological, breeding and cytogenetic parameters were used to assess species distinctiveness among accessions of *T. quartinianum*, *T. steudneri* and a distinct morphotype of *T. steudneri* (Accession 9452). The morphological characterizations showed that there was a considerable level of inter- and intraspecific variation among the accessions. The distinct morphotype exhibited a unique pattern of morphological characters. Hierarchical cluster analysis generated nine clusters and showed that the distinct morphotype was relatively closer to accessions of *T. quartinianum* than it was to *T. steudneri*. In fact, it stood even off the cluster it is grouped in. Principal component analysis was also performed and generated seven components to which the characters showed respective correlation.

Some level of interspecies compatibility was observed among some accessions of the involved taxa on the basis of interspecific hybridization. The results of the preliminary experiments showed that there were allowing conditions for the interspecific hybridization. Successful pollen tube growth, and compatibility between the time the pollen was shed and the stigma was receptive were some of the pre-fertilization barriers broken by some of the species combinations. Successful hybridization was, hence, attained in some of the intra- and interspecific crosses.

Each of the species and the distinct morphotype was observed to have a diploid chromosome number of 16. There was no difference in the chromosome number among these taxa. Chromosome number, hence, could not be used to identify and differentiate among the taxa.

Key Words: *Trifolium quartinianum, Trifolium steudneri*, distinct morphotype (Accession number 9452), Accessions, morphology, hybridization, cytogenetics.

# ACRONYMS

ACCN – accession

ACCNS - accessions

ANOVA – Analysis of Variances

DM - Distinct Morphotype

FCR – Flourochromatic Reaction Test

FDA – Flourescin diacetate

KMO test - Kaiser- Meyer- Olkin test

$K_3PO_4$ - Potassium phosphate

NaOH – Sodium hydroxide

PCA – Principal Component Analysis

SPSS - Statistical Package for Social Sciences

# 1. INTRODUCTION

## 1.1. General Characteristics of the Genus *Trifolium* L.

The genus *Trifolium* consists of annual or perennial herbs with 3-foliolate leaves. The leaves are usually characterized by toothed leaflets and stipules that are adnate to the petiole. The leaves are pinnately or palmately arranged with veins ending in teeth. The flowers occur in heads or short spikes and are variously coloured. In rare cases, solitary flowers are observed (Thonner, 1962; Zohary and Heller, 1984). The flowers bear five equal or unequal calyx lobes and persistent corolla. The upper filament is free while the other nine are united. The pod is surrounded by the corolla and calyx and bears from one to ten seeds (Thulin, 1982). The genus consists of 240 species (Zohary and Heller, 1984; Evans, 1962), out of which *Trifolium steudneri* and *Trifolium quartinianum* are the focus of this study. The genus has high forage potentials and nitrogen fixing ability (Akundabweni *et al.*, 1991).

## 1.2. The Taxonomic Position of the Genus *Trifolium* in the Plant Kingdom

Various classification schemes were used for the pea family. Melchoir (1964) included the genera *Parochetus, Trigonella, Factorovskya, Medicago, Melilotus* and *Trifolium* in the tribe *Trifolieae*. This tribe seems to be a natural group. The six genera under it do not constitute a phyletic group even though some morphological links are found within some of the genera (e.g. between *Medicago* and *Trigonella*). The following markers are used to classify the genera (Melchoir, 1964):

a) *Parochetus* Buch.-Ham. ex Don – is a monotypic genus, which occurs in the mountains of tropical Africa and tropical Asia. It is a perennial, creeping herb rooting at nodes. The flowers are blue-purple, axillary, solitary or 2-3 borne on common peduncle. The bracts are similar to the stipules at base of pedicels. The pods are many-seeded, linear and dehiscent. This genus is distinguished from the other five genera of this tribe by its caducous petals and digitate leaves.

b) *Trigonella* L. – This genus comprises some 135 species, which are distributed mainly in the Mediterranean and Irano-Turanian regions. A few also occur in the Saharo- Arabian and S. African regions. It consists of annual or perennial herbs. The flowers are variously colored, solitary or capitate. The bracts are inconspicuous. The Pods bear many seeds, and are linear, dehiscent or indehiscent.

c) *Factorovskya* Eig – One annual species E. Mediterranean. Stems are creeping and flowers are yellow, cleistogamous, geocarpus, penetrating into the ground by means of elongating peduncles and gynophores. The fruit is subterranean, ovate to globular, densely hairy, 1-2 seeded, indehiscent.

d) *Medicago* L. – About 100 species are included in this genus. They are mostly Mediterranean and Irano-Turranian with few Euro-Siberian and North African representatives. It encompasses both annual and perennial herbs. The flowers are small, yellow or violet, solitary or in few-flowered racemes or

heads. The bracts are either minute or absent. The pods are mostly coiled, many seeded, and indehiscent.

e) *Melilotus* Mill. – About 25 species are included in this genus. They are mostly Mediterranean and Irano-Turanian; a few Euro-Siberian. They include both annual and perennial plants. The flowers are small, yellow or white arranged in spike-like racemes. The bracts are inconspicuous or absent. The pods are globular or ovoid, as long as or somewhat longer than the calyx but not enclosed within it. They are indehiscent. The seeds in the pods are solitary or few.

f) *Trifolium* L. – This genus comprises about 240 species, which are mainly Mediterranean. Irano-Turranian, Afro-Alpine, Neotropic-Alpine, N.W. American and some Euro-Siberian representatives are also found. They can be either annuals or perennials. The leaves can be 3-, or rarely 5-9- foliolate. The flowers are variously colored and small to medium-sized. They are mostly arranged in heads or spike-like racemes, umbels or very rarely solitary. The bracts, when present, can be conspicuous or minute. Sometimes the inflorescence is involucrate by entire, dentate, or lobed stipules of upper leaves or by connate lower bracts. The pod is oblong or obvoid, usually enclosed within calyx but sometimes longer than the calyx. It is 1-2-, or rarely many-seeded. It is mostly indehiscent (Thonner, 1962; Lewis and John, 1965; Manning, 1965; Hutchinson, 1969; Rendel, 1979).

## 1.3. Ecology, Origin and Distribution of the Genus

There are two views about the center of origin of the genus *Trifolium*. The first view states that the Mediterranean region is the center of origin of the genus and the ancestors of the present day African species migrated southwards to east Africa. The other view, according to Norris (1956b) cited in Pritchard (1962), states that the east African region is the center of origin of the genus and northwards migration from the highlands gave rise to the species in the Mediterranean region. According to Zohary (1972), the origin of the genus is traced back to the ancestral section Lotodeae, which is assumed to have existed from Neogene times.

The genus is well represented in the Mediterranean region and in tropical Africa with a center of distribution in Ethiopia (Thulin, 1982). Zohary (1972) cited in Kahurananga (1987) identified Ethiopia as a secondary center of origin for the genus. Eeghen (1984; cited Harlan, 1969) also agreed that Ethiopia is a secondary center of diversity for the genus. The Ethiopian highlands are endowed with a rich diversity of locally adapted *Trifolium* species. It is reported that there are 32 species of *Trifolium* in Ethiopia, nine of which are endemic (Thulin and Hunde, 1989; Mugwira and Haque, 1991).

According to Kahurananga (1987), the genus occurs within the altitudinal range of 1450-3350 m above sea level. In general, the proportion of legumes in natural pasture increases with increasing altitude. There is a wide range of annual and perennial *Trifolium* species in these ranges of altitudes, particularly above 2200 m. Both *T. quartinianum* and *T. steudneri* grow in upland grasslands and bush lands, especially, in damp places. (Alemayehu Mengistu, 1987).

### 1.3.1. Altitudinal Ranges for the Growth of *T. quartinianum* and *T. steudneri.*

*T. quartinianum* grows from 1500-2600 m above sea level, while *T. steudneri* grows from 1100-2800 m above sea level (Thulin, 1989). Eeghen (1984) reported that *T. steudneri* grows even at altitudes as high as 3500 m above sea level. According to Kahurananga (1987), both species are found in the same altitudinal ranges, often growing together. The altitudinal range for the growth of these species is reported by other workers to be 1450-3500 m above sea level (Brown and Cochme, 1973; Gemechu, 1977). According to Alemayehu Mengistu (1975), the Ethiopian highland clover content is high, reaching up to 17% cover in some of the high altitude areas.

## 1.4. *Trifolium steudneri* Schweinf.

This species is an annual, glabrous herb up to 60 cm tall. The leaflets are narrowly elliptic and 40 by 8 mm in size. The petioles can reach up to 5 cm in length. The stipules have narrowly triangular tips. The inflorescences are globose, 10-20 flowered and 13- 20 mm across. Inflorescences are borne on long peduncles with several-nerved bracts forming an involucre. The outer bracts are abruptly and sharply pointed. They can reach up to 7 mm in length. The inner bracts are oblong and 3 mm long. The calyx is approximately 40 nerved, glabrous or occasionally pilose. The lobes are triangular, 2-3 mm long. The corolla is purplish and 7-8 mm long and sometimes with filiform tips, which characterize the species. The pods bear up to 2-4 seeds (Thulin, 1982).

### 1.5. *Trifolium quartinianum* A. Rich.

This species is an annual sub-glabrous herb up to 60 cm tall. The leaflets are narrowly oblong and up to 52 by 16 mm in size. The leaflets bear petioles that can be 4 cm long and stipules with or without triangular tips. The inflorescences are globose, long, and pedunculate with several nerved bracts that form an involcre. The outer bracts are ovate, lobed or toothed and up to 7 mm long. The calyx is approximately 40- nerved; lobes 4-7 mm long, ovate and overlapping at the base, drawn out into subulate points. The corolla are purplish red, rarely white and 11-12 mm long. The pod usually bears 4-5 seeds (Thulin, 1982).

### 1.6. Key Characteristics of the Genus and the two Species

The key characteristics of the genus *Trifolium* include the persistent corolla and the pod, which is less than twice as long as the calyx and 1 to 9 seeded. The petals, after flowering, are persistent and the claws of the wings and keel are attached to the staminal tube. The pods are straight, short, and enclosed in the persistent corolla. The differentiating characteristics between *T. steudneri* and *T. quartinianum* include the length of the calyx lobes and the corolla. In *T. quartinianum,* the calyx lobes are 4-7 mm long while they are 2-3 mm long for *T. steudneri*. The length of the corolla, on the other hand, is 11-12 mm long in *T. quartinianum* and 7-8 mm in *T. steudneri* (Thulin, 1982).

## 1.7. Cytology

Karpechenko (1925; cited in El-kholy, 1990) reported the chromosome numbers of *Trifolium* for the first time. Wexelsem (1928; cited in El-kholy, 1990) studied chromosome variations in *Trifolium* and stated that chromosome morphology cannot be used for classification, because there are high levels of similarity in chromosome morphology among unrelated species.

Among notable contributions to the cytology of *Trifolium* species are: Britten (1963), who surveyed the chromosome numbers of 71 species; Pritchard (1962, 1967 and 1969), who reported the chromosome numbers for 31 species, 18 of them for the first time; Putiiesky and Katznelson (1970), who investigated the chromosome numbers and generic systems of 12 species; and Chen and Gibson (1975) who reported the chromosome numbers and karyotypes of 15 species.

Zohary and Heller (1984) restated the basic chromosome numbers in 80% of the examined species to be x = 8. Other basic numbers were also described. These include: x = 7 (in 15%), x =6 (in 2%) and x = 5 (in 3%). While x = 8 has been recorded for 46 % of the annuals, x = 7 plants are mostly annuals and counts of x = 6 and x =5 were noted only for Mediterranean annuals (Zohary and Heller, 1984).

Pritchard (1962) observed a large variation in chromosome size between the chromosomes of each species he examined. There was also variation in the absolut sizes of the chromosomes. Out of the 14 species examined, only few wer polyploids. These include *T. africanum* (2n = 32), *T. burchellianum* va *burchellianum* (2n = 48) and *T. burchellianum* var. *johnstonii* (2n = 96). All the re

have a diploid chromosome number of 16. In most of the species, a gradual decrease in size of the chromosomes was observed. Accordingly, Senn (1938; cited in Pritchard, 1962) stated that chromosome size could play a major role in chromosomal evolution in the *Leguminosae*. Stebbins (1950; cited in Pritchard, 1962) agreed to this saying that differences in chromosome size may suggest a general trend of increase in specialization. This, however, does not preclude an opposite trend in any part of the family in which a phylogenetic trend towards smaller chromosome size is observed.

El-kholy (1990) reported that *T. steudneri* has a pair of large telocentric SAT-chromosomes (which is the largest) and small sub-metacentric chromosomes, whereas, *T. quartinianum* has two pairs of telocentric, four pairs of sub-metacentric, one pair of large metacentric and another pair of telocentric chromosomes with satellite almost as large as the larger arm. According to him, both species have a diploid chromosome number of 16. Pritchard (1962) stated that the karyotype of *T. steudneri* is associated with a high degree of specialization. The chromosomes of this species have almost sub-terminal centromeres with large gradients in size. He suggested that this species might be an offshoot from the main body of African clovers.

## 1.8. Breeding Systems and Interspecific Hybridization

*Trifolium* has a typical papilionaceous flower suitable for insect pollination. The insect vectors visiting the flowers include the honeybee, bumble bee and some *Lepidoptera*. These insects have long proboscises and visit the flowers to collect nectar and pollen. The insects apply pressure on the standard of the flowers. This makes the stigma and the anther to protrude from the keel. The stigma and the anther revert to their former position when the pressure is relieved. Thus, the anthers and stigma are pressed against the underside of the insect's head and pollination occurs. The pistil is usually longer than the stamens and slightly curved. The anthers are dehiscent even in bud. The nectar is secreted under the base of the stamens and collects at the base of the corolla tube (Zohary and Heller, 1984).

Most species of the genus are allogamous and the best seed-set results from cross-pollination with another plant of the same species. Certain species are autogamous and although the flowers do open normally, pollination occurs spontaneously prior to this event. However, some autogamous species need to be tripped before setting seed. The flowers of some species, such as *T. baccarinii* and *T. lanceolatum*, do not open during blossoming. No bees or other pollen-bearing insects were observed on such cleistogamous flowers. The pollen of their dehiscing anthers reaches the stigma. In some cases, the pollen may germinate while still in the anther, the pollen tubes piercing through the wall of the anther on their way to the stigma (Zohary and Heller, 1984).

Both *T. quartinianum* and *T. steudneri* are annuals and self-pollinating (Kahurananga, 1987). It is reported that *T. quartinianum* needs 80-120 days to flower, after planting. *T. steudneri*, on the other hand, needs 67-93 days to flower (Kahurananga and Asres Tsehay, 1983).

Studies on the breeding system of some clovers showed that the annual *T. steudneri* is autogamous (Pritchard and Mannetje, 1967). Some perennial species are known to be partially self-sterile. For example, *T. fragiferum*, in its region of origin, the Mediterranean, is a self-incompatible species, but with occasional self-compatible plants (Davis *et al.*, 1966).

The first fertile interspecific hybrid was obtained by Brewbaker and Keim (1953). This cross involved *T. repens* and *T. nigrescens*. Some interspecific hybridizations are also reported in the genus. Ascherson and Graebner (1906-1910; cited in Evans, 1962), for example, showed the presence of some natural hybrids. Intensive investigations by Guravich and Trimble (1949; cited in Evans, 1962) resulted in a few shriveled seeds in some species combinations. Other successful hybrids were also reported by Pandey (1957) between *T. repens* and *T. nigrescens* and Taylor (1959) between *T. pratens* and *T. desmissum*. Wexelsen (1928; cited in Evans, 1962), however, attempted to cross nine *Trifolium* species in 18 combinations, but did not obtain a single hybrid plant. Evans (1962) also performed experiments on interspecific hybridizations and found that the genus shows a high degree of cross-incompatibility.

## 1.9. Pollen viability

Interspecific hybridizations are greatly hampered by reduced pollen viability and inhibition of the pollen tube growth in the style. These are important pre-fertilization barriers that prevent interspecific hybridizations in the genus *Trifolium* (Evans, 1962). Sorensen and Nagahara (1989) stated that the maintenance and testing of pollen grain viability is an important aspect of plant breeding. Pollen grains need to be of high quality (pollen with high rates of germination and fertilizing ability) to be able to germinate on the stigma. The quality of pollen grains is affected both by the physiological status of the parent plant and the weather conditions. Different species have different life spans for their pollen. The life span of pollen grains under natural conditions varies from a few hours to more than a year (Frankel and Galun, 1977).

Various methods were devised to determine whether a pollen grain can produce a pollen tube and fertilize the ovules or not. The most direct way is to see whether the pollen effectively induces the ovary to grow and a fruit to develop when placed on a stigma. Another is to see whether the pollen germinates when placed on a stigma. A third is to see whether the pollen grows *in vitro* (Pacini *et al.*, 1997). There are also indirect methods of determining pollen viability. These methods are based on cytological parameters. One is the fluorochromatic reaction (FCR) test, which determines the state of the pollen plasma membrane (Heslop-Harrison and Heslop-Harrison, 1970; Shivanna and Johri, 1985; Shivanna *et al.*, 1991). It is used to monitor pollen viability. The FCR test reflects the competence of the pollen grain to perform its normal function in the pistil. Viability of the pollen grains shows correlations with potential pollen germinability (Dafni, 1992; Diggle *et al.*, 2002). Shivanna and Johri (1983) stated that the FCR test could be used to assess the intact condition of the plasma lemma and

the activity of esterase. The enzyme (Esterase) cleaves the fluorescin ester, fluorescin diacetate.

The FCR test can be used both for binucleate and trinucleate pollen grains. But since the three celled pollen grains are difficult to germinate *in vitro*, it is difficult to determine their viability in this way (Shivanna *et al.*, 1991). For some species, the FCR test does not seem to be an appropriate method to test pollen viability. In *Leucaena*, for example, both the dead and live pollen grains fluoresce equally making it difficult to determine pollen viability based on the FCR (Sorensson and Nagahara, 1989).

The effect of temperature on pollen germination and growth is variable. In some species high temperatures (30°C) adversely affected pollen germination and low temperature (20°C) in others. A high humidity (93-98% RH) was essential for good pollen germination and growth. Good pollen tube growth usually accompanied good germination; however, pollen from *T. rueppellianum* var. *lanceolatum* grew best at temperatures below 25°C although germination was possible over the whole range of temperatures used. Relative humidity (RH) and extreme temperatures affect pollen vigor and pollen viability. RH is a very important factor in this respect. It is reported that a high RH of 93-98% is essential for good pollen germination and growth in *Trifolium* species (Pritchard and Mannetje, 1967).

A pollen viability test can be generally used to evaluate the fertility of a parent plant, to monitor the state of the pollen to be used for storage, to evaluate the chance of pollen germination in studies of pollen-stigma interaction and to study incompatibility and fertility between species (Dafni, 1992).

## 1.10. Stigma Receptivity

Effective fertilization of ovules dependens on the receptivity of the stigma and the capability of the stigma to allow hydration of pollen. This, in turn, allows the penetration of the styles by the pollen tubes. The receptivity of the stigma declines if it encounters water loss or infection by pathogens. The stigma is often protected or exposed briefly.

In Papilionoideae, the stigma is enclosed in the keel petals. Generally, the receptivity of the stigma is highly dependent on the physiological condition of the parent plant and the intra-floral microclimate (Corbert, 1990). These include the age of the flower, the time of the day and the presence or absence of stigmatic exudates. Stigma receptivity greatly influences the rate of self-pollination. The duration of stigma receptivity varies from a few hours to ten days (Dafni, 1992).

## 1.11. Pollen Tube Growth

Pollen development can be considered as an ideal system not only for the analysis of important processes in plant reproduction, but also for studying different aspects of plant biology such as cell fate determination, cellular differentiation, intercellular and intracellular signaling and polar growth (Procissi *et al.*, 2001).

Pollination, in flowering plants, starts when pollen lands on the stigma. The process continues as the pollen germinates and grows through intracellular spaces in the pistil, and it culminates when the pollen tube reaches the ovary and fertilization occurs. This complex process involves many interactions, including cell-cell recognition and intracellular and intercellular signaling, as well as many other factors that remain to be identified. Events occurring during pollination are assumed to be under tight genetic and cellular control because pollen tube

growth needs to be regulated both spatially and temporally. Pollen tubes are a highly specialized cell type, comprising a generative cell, which contains the two sperm cells and a vegetative nucleus. Thus, a pollen tube in fact contains a cell within a cell, which is itself haploid. Pollen tubes reaching the bottom of the pistil and the ovaries must utilize a complex guidance system as they twist and turn to gain entrance to the ovules through the micropyles. It is currently believed that chemotropic signals play a major role in guiding pollen tubes toward the ovules at this stage (Tong. 1999).

The success of interspecific hybridizations is dependent on the growth of the pollen tube down the style. Failure of the pollen tube to grow down the style is one pre-fertilization incompatibility barrier to interspecific hybridizations. The growth of the pollen tube down the style, thus, can be used as an indicator to rule out this pre-fertilization barrier of interspecific hybridizations (Tong. 1999).

This study will be directed towards pinpointing morphological characters that can be used to differentiate among the two species and the distinct morphotype. The taxonomic identity of the distinct morphotype will be assessed using morphological and hybridization-based parameters.

# 2. OBJECTIVES

## 2.1. General Objectives

- To find morphological differences between *T. quartinianum* and *T. steudneri,*
- To determine whether the distinct morphotype is *T. quartinianum* or *T. steudneri,*
- To study the breeding system of the three taxa.

## 2.2. Specific Objectives

- To study morphological characters used to differentiate among the three taxa.
- To assess pollen viability, flower development and pollination behavior,
- To asses whether the two species are able to hybridize or not,
- To screen the isolating mechanisms for the out-breeders.

# 3. MATERIALS AND METHODS

Seeds collected from various places in Ethiopia were used for the experiment. The following accessions were selected for *T. steunderi:* 9058, 10125, 9720, 8485, 9956, 7658, 9991, 8461, 7659 and 9966. The accessions selected for *T. quartinianum* were: 9455, 8535, 11056, 7693, 13808, 13716, 10059, 8452, 6297 and 6301 (ILRI gene bank accession numbers). Accession number 9452 represents the distinct morphotype. The accessions that were used, the place of collection and the ILRI gene bank accession numbers are summarized in table 1.

**Table 1. Accessions Used and Place of Collection**

| Accession no. | Species | State | Region | Elevation |
|---|---|---|---|---|
| 6297 | *Trifolium quartinianum* | Gojam | Amara | 2380 |
| 7693 | *Trifolium quartinianum* | Gojam | Amara | 2360 |
| 6301 | *Trifolium quartinianum* | Gojam | Amara | 2450 |
| 8452 | *Trifolium quartinianum* | Kefa | Oromia | 1740 |
| 8535 | *Trifolium quartinianum* | Gojam | Amara | 2100 |
| 9455 | *Trifolium quartinianum* | Gojam | Amara | 1950 |
| 10059 | *Trifolium quartinianum* | Welega | Oromia | 1720 |
| 13716 | *Trifolium quartinianum* | Shewa | Amara | 2010 |
| 7658 | *Trifolium steudneri* | Gojam | Amara | 2450 |
| 7659 | *Trifolium steudneri* | Gojam | Amara | 2510 |
| 8461 | *Trifolium steudneri* | Kefa | Oromia | 1950 |
| 8485 | *Trifolium steudneri* | Shewa | SNNP | 2120 |
| 9058 | *Trifolium steudneri* | Gojam | Amara | 2420 |
| 9720 | *Trifolium steudneri* | Shewa | Oromia | 2880 |
| 9956 | *Trifolium steudneri* | Shewa | SNNP | 2120 |
| 9966 | *Trifolium steudneri* | Shewa | SNNP | 2000 |
| 9991 | *Trifolium steudneri* | Kefa | Oromia | 1750 |
| 10125 | *Trifolium steudneri* | Shewa | Amara | 2320 |
| 9452 | *Trifolium steudneri* (DM) | Gojam | Amara | 1850 |

Ten accessions of each of the two species and an accession of the distinct morphotype were scarified on sand paper to break the hard seed coat. They were placed on wet filter paper in Petri dishes. This allows the seeds to absorb water and germinate. They were then placed in the cold room at 8°C for five days. They started to germinate on the fifth day and the germinating seeds were transferred to soil in 4-inch pots.

The accessions were selected to represent accessions from several ecological ranges in Ethiopia. This was done so as to represent the overall distribution of the species in Ethiopia. The seedlings were kept in the screen house until they became stronger. They were watered twice a day using sprinklers. When they became stronger, they were taken to a green house at ILRI. They were given plant food, once a week, starting the fourth week after planting.

## 3.1. Morphology

A great deal of morphological similarity exists among the involved taxa. The study focused on pinpointing key morphological characteristics, which could be used to differentiate among the taxa. For the morphological studies, seeds were sown and seedlings transferred to pots. Five plants of an accession were planted and used for as replicates according to CRD (completely randomized design) for measuring the morphological characters. Each of these plants was grown on a separate pot. The characters under consideration were measured for all the accessions at peak flowering. The results from the two species were compared with the results of the

1. **Length of the corolla (LOC)** – the length of the corolla (cm) from its base to its tip.

2. **Length of the calyx–lobe (LCL)** - the length of the calyx-lobe (cm) from its base to its tip.

3. **Presence or absence of leaf marks (lfmark)** - the presence (1) or absence (0) of leaf marks on the leaves.

4. **Plant Height (PHT)** - the height of the plant from the ground to the tip of the plant is measured in cm.

5. **Hundred seed weight (HSW)** - the weight of hundred seeds measured in gm.

6. **Nature of the stipule (NS)** - the nature of the margin of the stipule is described as smooth (0) or serrated (1).

7. **Length of the terminal leaflet (LTL)** - the length of the terminal leaflets from the base to the tip measured in cm.

8. **Length of the side leaflets (LSL)** - the length of the two side leaflets was measured from their bases to their tips in cm.

9. **Width of the terminal leaflet (WTL)** - the width of the terminal leaflet was measured at the widest point in cm.

10. **Width of the side leaflets (WSL)** - the width of the two side leaflets was measured at their widest points in cm.

11. **Number of pods per inflorescence (NPI)** - the number of pods per inflorescence.

12. **Number of seeds per pod (NSP)** - the number of seeds per pod.

13. **Leaflet length to width ratio of the terminal leaflet (LWRT)** –the ratio of the length and the width of the terminal leaflet was computed.

14. **Leaflet length to width ratio of the two side leaflets (LWRS)** - the ratio of the length and the width of the two side leaflets was computed.

15. **Direction of the leaf serration (DLS)** - the direction of the serration of the leaf margin was described as forward (1) and perpendicular to the leaf margin (0).

## 3.2. Emasculation and Bagging

Five flowers were used for cross-pollination and emasculated by removing the anthers before they dehisced. The emasculation was done using forceps. This helps in carefully picking undehisced anthers. The emasculated flowers were, then, used as female parents and were cross-pollinated using pollen grains taken from anthers of other plants (in case of intraspecific hybridization), or the same plant, but different flowers in case of selfing.

The pollination was done using pollinating brushes to bring enough pollen from the dehiscing anthers (Williams, 1954; Hutton, 1954; cited in Pritchard and Mannetje, 1967). To avoid the risk of contamination by pollen from unwanted sources, the flowers were covered by plastic bags that prevent pollen entrance.

Five flowers were bagged (i.e. covered with pollination bags) and allowed to self-fertilize to see the effect of bagging on the outcome. Some plants were left untouched to serve as controls. The number of pods per flower and the number of seeds per pod

were counted and compared both between and within accessions for the plants selfed with- and with out- bagging.

### 3.3. Pollen Viability

The pollen viability test was performed by using the Fluorescin Diacetate (FDA) solution. The FDA was prepared by the following procedure:

1. 0.01 gm of FDA was first dissolved in 10 ml of acetone,
2. 0.5 M sucrose was prepared by dissolving 17.1 gm of sucrose in 100 ml of water,
3. 0.1 ml of FDA in acetone was mixed with the sucrose solution
4. The final solution ($10^{-6}$ M FDA) was used as a working solution (Kasten, 1984).

Pollen grain viability was assessed under natural condition using the FCR test. For this experiment, flower buds that were in the verge of opening were selected and tagged. Five buds from each of the plants from each of the accessions were used at different times. Microscopic slides, fine forceps and the FDA working solution were taken to the green house where the plants were kept.

Two drops of the solution were placed on each slide. Few pollen grains were carefully picked at a time from individual buds using forceps and mixed with the solution on each slide. These were taken to the laboratory and pollen grains were checked for viability using a fluorescence microscope. The viability of pollen grains from each flower was tested four times (at 8: 30 am, 12 am, 2 pm, and 4 pm) during a day to know how long the pollen grains on each flower remain viable after dehiscence.

### 3.4. Stigma Receptivity Test

The size of the flowers at which the stigma was receptive was measured. When the stigma is receptive, it becomes shiny. This feature was used to determine the sizes of the flowers associated with the stigma receptivity. Five flower buds of varying size were collected and observed to describe the size that corresponds to when the stigma is receptive. The corresponding sizes of the buds were measured in cm using ruler to obtain some idea about incompatibility before making interspecific hybridizations.

### 3.5. Pollen Shedding Test

Five flowers were used per accession to study the sizes of the flowers associated with pollen shedding. The anthers dehisce at certain periods of the day. The time at which the pollen is shed and the stigma is receptive should be synchronized. This synchrony, in addition to other mechanisms, supports successful interspecific hybridization. The time of the day at which the pollen is shed was recorded by looking at different flowers at different stages of development. The size of the flowers when the pollen was shed was recorded by looking at the dehiscing anthers using hand lenses (Pacini *et al*., 1997).

## 3.6. Reciprocal Crossing

Seeds were germinated on wet filter paper and sown for the reciprocal crossing. The seeds were sown in two phases. This is because *T. quartinianum* needs 80 to 102 days to flower after sowing. *T. steudneri,* on the other hand, needs 67-93 days to flower (Kahurananga and Asres Tsehay, 1983). The distinct morphotype was reported to flower three weeks later than the rest of the accessions. The first phase of planting was for those accessions of *T. quartinianum* and *T. steudneri* excluding the distinct morphotype (9452). The second phase, on the other hand, was for all accessions including the distinct morphotype (9452). This phase of sowing was done three weeks after the first phase.

Three weeks after the flowering of the accessions of *T. quartinianum* and *T. steudneri,* the distinct morphotype flowered. In the interim, reciprocal crossing was done among the early flowering accessions of *T. quartinianum* and *T. steudneri.* Later on, reciprocal crossing, which involved all the accessions, was done. For each of the accessions, five plants were used.

## 3.7. Pollen Tube Growth Assessment

Pollinated styles were fixed in a 1:8:1 (by volume) solution of formalin: alcohol: acetic acid for about 24 hours. After rinsing in tap water, they were treated in a nearly saturated aqueous solution of sodium hydroxide (8N) for 24 hours. This clears and softens the tissue, and permits adequate penetration of the dye. The softened styles were transferred to a small beaker of tap water for one hour or more to remove most of the sodium hydroxide. Staining is accomplished next in a 0.1% solution of water-soluble aniline blue dye in 0.1 N $K_3PO_4$ for 4 hours. The styles were, then, mounted in a few drops of the staining medium on clean glass

slides and are covered with cover slips. This makes the callose in the pollen grains and tubes to be stained and to fluoresce brightly under short-wave light.

The preparation was observed under fluorescence microscopes for the pollen tube growth (Martin, 1959; Kalinganire *et al*., 2000; Buide and Guitian, 2002,). The growth of the pollen tube down the styles is traced by the callose that lines these tissues down the style.

## 3.8. Cytology

### 3.8.1. Preparation of Mitotic Chromosomes from Plant Root Tips

The tissues that have high rates of division, and thus used for the experiment, were the root tips of the plants. The root tips were obtained by germinating seeds on wet filter papers. The radicles were immersed in a 0.05% aqueous colchicine solution for 3-5 h at room temperature, or cold treated by storing them in water at $1^0$C for 24 hours. They were then fixed in a 3:1 alcohol: acetic acid fixative for about 1 hour. Maceration was performed in an aqueous solution of 4% cellulase and 4% pectinase ($p^H$ 4-4.5), at 36-$37^0$C for 1h. The enzyme solution was decanted and the roots were rinsed with distilled water. The remaining root tips were, then, stained by aceto-orcein and squashed under a cover slip by gently tapping the cover slip using pencil erasers (Pritchard, 1962; Jones and Richards, 1991; Kifle Dagne and Heneen, 1992; Hills *et al*., 1996). The preparations were then observed under microscopes to find spread metaphase chromosomes; C- metaphase.

### 3.9. Statistical analysis

The data was analyzed using ANOVA, paired samples T- test, non-parametric test (Binomial test), Pearson's correlation, scatter plots, Principal Component Analysis and cluster analysis. SPSS (Version 10.0, 1999) was used to help in the analysis.

Test of homogeneity of variances was calculated before undertaking ANOVA. ANOVA was computed following and according to the results of the test of homogeneity. When there was homogeneity among the variances, ANOVA was computed using Scheffe (which assumes equal variances among groups). When, on the other hand, the group variances were not equal, ANOVA was computed using Tamhen's T2 that assumes unequal variances. The comparisons were made between the distinct morphotype and accessions of the two species, among accessions of the same species and between the two species.

Binomial test was also performed at a test proportion value of 0.5. This value was used assuming that the characters were distributed randomly with equal probability of occurrence. For one sample T- test, the mean values of the distinct morphotype were taken as references and were compared with the mean values of accessions of *T. quartinianum* and *T. steudneri*.

# 4. RESULTS

## 4.1. Morphology

### 4.1.1. Plant height

*T. quartinianum* accessions

Accession 9455 was the tallest (44.0 cm) accession, while 8452 was the shortest (22.0 cm). The average plant height was 34.5 ± 11.2 cm for this species. The least observed variability was 0.58 (13808), while the largest was 16.9 (for 6301). The average (mean ± SD) plant height for all the accessions is listed in Appendix 1 and Figure 1. Test of homogeneity at $P< 0.05$ witnessed that the group variances were not homogenous ($P= 0.000$). One way ANOVA showed that there was a significant ($P= 0.000$) difference among the means of the *T. quartinianum* accessions at $P< 0.05$. One sample T-test was also performed among the accessions of *T. quartinianum* and the distinct morphotype. The T-test showed that there was significant difference ($P= 0.000$) both at $P< 0.05$.

*T. steudneri* Accessions

The distinct morphotype (accession 9452) was the tallest (45.0 cm) within this species. Accession 8461, on the other hand, was the shortest (30.5 cm). The average plant height was 36.2 ± 5.29 cm. The average height scored by members of the distinct morphotype was 45.0 ± 1.00 cm. Accession 8485 showed the highest variability (6.0). The distinct morphotype, on the other hand, exhibited the least (1.00) (Appendix 1 and Figure 1). The group variances were not homogenous as was showed by test of homogeneity of variances ($P= 0.001$) at $P< 0.05$. ANOVA was computed and showed that there was significant ($P= 0.000$) difference among the means at $P< 0.05$. A significant difference was obtained when one sample T- test was performed on the *T. steudneri* accessions and the distinct morphotype at $P< 0.05$.

Accessions of the two species were compared with each other so as to see whether these species were distinct from each other or not. Test of homogeneity performed on these groups showed (P= 0.000) that the variances were not equal. ANOVA calculated on the two groups showed (P= 0.014) that the two species had significant difference at P< 0.05 (Appendix 2).

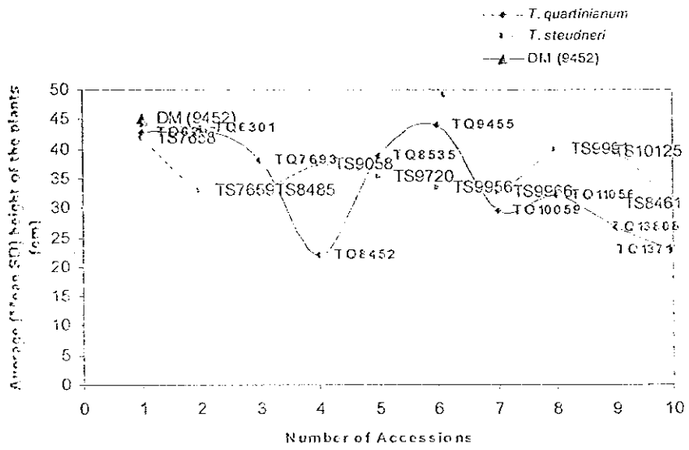


Figure 1. Average (Mean ± SD) height of the plants (cm).

## 4.1.2. Hundred Seed Weight

### *T. quartinianum* accessions

The average hundred seed weight for *T. quartinianum* accessions was 0.15 ± 0.02. The accession with the largest hundred seed weight was 13808 (0.191). The lowest hundred seed weight was recorded for accessions 8452 and 8535 (0.14). The highest variability (0.016) was observed in accessions 8452, 8535 and 9455; whereas the smallest (0.00005), in 6301 (Appendix 1 and figure 3). One way ANOVA was calculated and showed that there was significant difference (P= 0.000) among the accessions at P<0.05. A significant difference (P= 0.000) was observed between the distinct morphotype and accessions of *T. quartinianum* when one sample T-test was calculated at P< 0.05.

## *T. steudneri* accessions

The average hundred seed weight of the accessions of this species was 0.17 ± 0.017. Accession 7658 had the largest hundred seed weight (0.153), while the distinct morphotype (9452) had the lowest (0.0988). The distinct morphotype exhibited the smallest variability (0.00005) while accession 9966 showed the largest variability (0.013) of the group. Appendix 1 and figure 4 summarize the result. One sampled T- test was performed to see if there existed significant difference between the distinct morphotype and accessions of *T. steudneri*. When one way ANOVA was computed, significant difference (P= 0.000) was observed among the involved accessions at P< 0.05. The group variances were showed to be non- homogenous (P= 0.000) by test of homogeneity of variances at P< 0.05.

All the accessions were pooled together and test of homogeneity of variances was performed on them and the distinct morphotype. The test, at P < 0.05 and P< 0.01, showed that there was no homogeneity (P = 0.007) among the means of the accessions. ANOVA showed that there was a significant (P= 0.000) difference among the means at P< 0.05. The two species were compared to each other using ANOVA at P< 0.05. Test of homogeneity of variances at P< 0.05 showed that the variances were homogenous (P= 0.099) at P< 0.05. The two species were found to had significant difference (P= 0.000) with each other (Appendix 2).

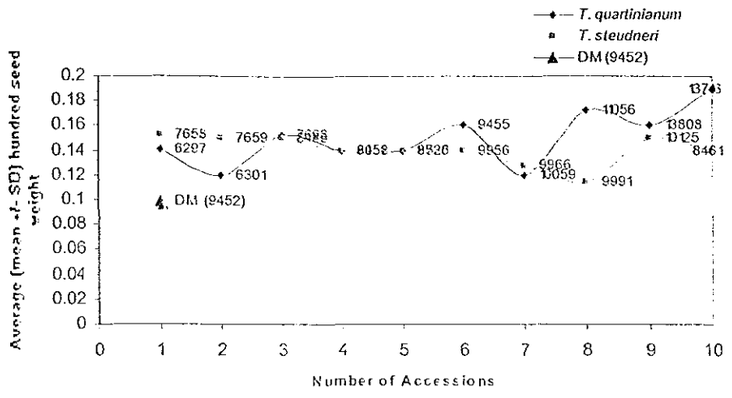


Figure 2. Average (Mean = SD) hundred seed weight

### 4.1.3. Average length of the terminal leaflet

*T. quartinianum* accessions

The length of the terminal leaflet was measured for all the taxa. Accession 13808 had the longest (5.46 cm) length of the terminal leaflet. The shortest was accession 8535 (3.94 cm). The largest deviation was 1.08 and was recorded in accession 8452. The lowest deviation recorded, on the other hand, was 0.09 and was for accession 10059. The average length of the terminal leaflet was 4.66 ± 0.72 cm for the species (Appendix 1 and figure 5). One way ANOVA and test of homogeneity were performed which showed that there was a significant difference (P= 0.000) among the means and the variances were not equal (P= 0.000) at P< 0.05. One sample T- test witnessed significant difference (P= 0.000) among the means of the accessions of this species and the distinct morphotype at P< 0.05.

## *T. steudneri* accessions

Accession 7658 had the highest score (5.4 cm) of the terminal leaflet length, while 8485 scored the least (3.7 cm). The average length for all the accessions was 4.47 ± 0.64 cm. The distinct morphotype scored an average length of 4.18 ± 0.43 cm. The highest deviation observed was 0.61 and was for 9956. Two accessions, 9991 and 9720, had the lowest deviation, 0.27. Appendix 1 and Figure 6 summarize the average values for this species. One-way ANOVA showed that there was significant (P= 0.000) difference among the means while test of homogeneity witnessed that the group variances were homogenous (P= 0.704) at P< 0.05. One sample T- test was also performed which showed that there was a significant difference (P= 0.001) among the means at P< 0.05.

Test of homogeneity of variances was performed at P< 0.05 and showed (P= 0.306) that there was homogeneity. The two species were found not to have significant difference (P= 0.263) from each other (Appendix2).

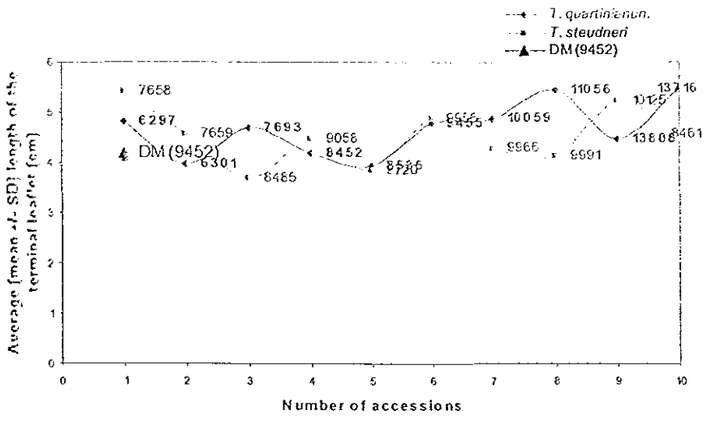


**Figure 3. Average (Mean ± SD) length of the terminal leaflets of all accessions**

### 4.1.4. Average width of the terminal leaflet

*T. quartinianum* accessions

The width of the terminal leaflet was measured at half way from the leaf base. The largest mean (1.48 cm) was recorded for 6301, whereas the smallest mean (0.74 cm) was that of 10059. The highest and the lowest deviations were 0.45 (for 9455) and 0.04 (for 7693) respectively. The average value for this species was 1.17 ± 0.32 cm (Appendix 1 and Figure 7). A significant difference (P= 0.031) was observed between the accessions of *T. quartinianum* and the distinct morphotype when one sample T- test was performed at P< 0.05. The group variances weren't homogenous as is witnessed by test of homogeneity (P= 0.006) at P< 0.05. ANOVA showed significant (P= 0.000) difference among the means of the accessions.

*T. steudneri* Accessions

The average leaflet width for the accessions of *T. steudneri* was 1.02 ± 0.18 cm. The distinct morphotype had the largest measurement (1.40 cm), while 9720 scored the least (0.78 cm). The highest deviation was 0.24 and was recorded for 8485. The lowest deviation was 0.04 (Accessions 9058 and 9720) (Appendix 1 and Figure 8). One sample T- test showed that there was significant difference (P= 0.000) between the *T. steudneri* accessions and the distinct morphotype at P< 0.05. One way ANOVA and test of homogeneity were performed on *T. steudneri* accessions and indicated that there was significant difference (P= 0.000) and inequality (P= 0.02) in the group averages and variances at P< 0.05, respectively.

Test of homogeneity was calculated on the combination of the three taxa and showed that the variances were not equal (P= 0.000) at P< 0.05. ANOVA was also calculated and showed that there was significant difference (P= 0.000) among the means at P< 0.05. Test of homogeneity

of variances was calculated for the two species at P< 0.05 and showed that the group variances were not (P= 0.000) homogenous. ANOVA, at P< 0.05, showed that the two species showed significance difference (P= 0.000) from each other (Appendix 2).

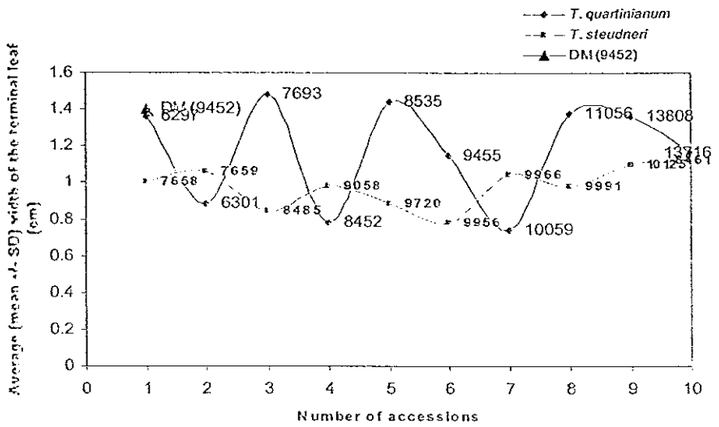


Figure 4. Average (Mean ± SD) width of the terminal leaflet (cm)

### 4.1.5. Average length of the two side leaflets

*T. quartinianum* accessions

The largest average length (5.18 cm) of the side leaflets was recorded for 10059, while the lowest (3.56 cm) was that of 8452. Accession 13716 had the largest variability (0.96). The smallest variability (0.23) was recorded for 8535. The average leaflet length for this species was 4.34 ± 0.71 cm (Appendix 1 and figure 9). A significant difference (P= 0.014) was observed between the means of *T. quartinianum* accessions and the distinct morphotype when one sample T- test was performed on the accessions at P< 0.05. One way ANOVA and test of homogeneity were calculated for these accessions. ANOVA (at P< 0.05) showed that there was significant difference (P= 0.000) among the means. The variances of this group of accessions were found to be unequal (P= 0.016) by test of homogeneity at P< 0.05.

## *T. steudneri* Accessions

Accession 7658 had the largest average length (5.09 cm) for the side leaflets, while the distinct morphotype had the lowest (3.79 cm). The mean leaflet length for this species was 4.40 ± 0.58 cm. The largest variability (0.65) was observed for 10125, whereas the smallest (0.25) was observed for 9452 (the distinct morphotype). Appendix 1 and Figure 10 summarize the result. One sample T- test showed that there was significant (P= 0.000) difference between the means of *T. steudneri* accessions and the distinct morphotype at P< 0.05. ANOVA showed significant difference (P= 0.000) among the accessions at P< 0.05. The group was proved homogenous (P= 0.270) by test of homogeneity of variances at P< 0.05.

Test of homogeneity was performed for the combined accessions and showed that the group was no homogeneity (P=0.002) among the variances at P< 0.05. One way ANOVA was showed that there was significant difference (P=0.000) among the means at P< 0.05. ANOVA showed that the two species were significantly different from each other (P= 0.01) (Appendix 2).

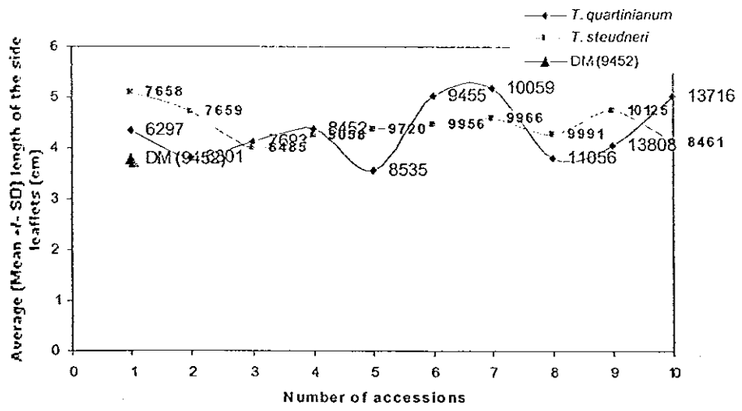


**Figure 5. Average (Mean ± SD) length of the side leaflets**

### 4.1.6. Average width of the two side leaflets

*T. quartinianum* accessions

The width of the side leaflets was measured at their widest points. The average leaf width for the species was found to be 1.02 ± 0.23 cm. The highest and the lowest averages were 1.27 cm (that of 6301) and 0.71 cm (that of 8452) respectively. The largest recorded variation was 0.26 (for 11056), while the smallest was 0.06 and was for 8452. Significant difference was found in the group (P= 0.000) when one-way ANOVA was computed at P< 0.05. Test of homogeneity (at P< 0.05) was also computed and showed that the group variances were not homogenous (P= 0.006). One sample T- test showed significant (P= 0.000) difference between the leaflet width of the distinct morphotype and that of *T. steudneri* accessions.

*T. steudneri* Accessions

Accession 9452 scored the highest (1.24 ± 0.08 cm) average width of the leaflets of the *T. steudneri* accessions. The smallest width (0.79 ±0.09 cm) was recorded to 9058. The average width of the leaflets of this species was 0.93 ± 0.15 cm. The largest variation (0.13) was observed in accession 10125, whereas the smallest (0.04) was observed in accession 7658 (Appendix 1 and figure 12). One sample T-test showed a significant (P= 0.000) difference between the means of the accessions of *T. steudneri* and the distinct morphotype at P< 0.05. ANOVA and test of homogeneity were computed for all the accessions and witnessed the presence of significant difference (P= 0.000) and unequal variances (P= 0.003) in this group at P< 0.05.

Test of homogeneity of variances, calculated for the combined accessions, showed that the variances were not homogenous (P= 0.000). A significant (P= 0.000) difference was shown among the accessions of the three taxa by ANOVA at P< 0.05. Test of homogeneity of

variances witnessed that the variances were not homogenous (P= 0.001) at P< 0.05. ANOVA at P< 0.05 showed that there was significant difference (P= 0.000) between the two species (Appendix 2).

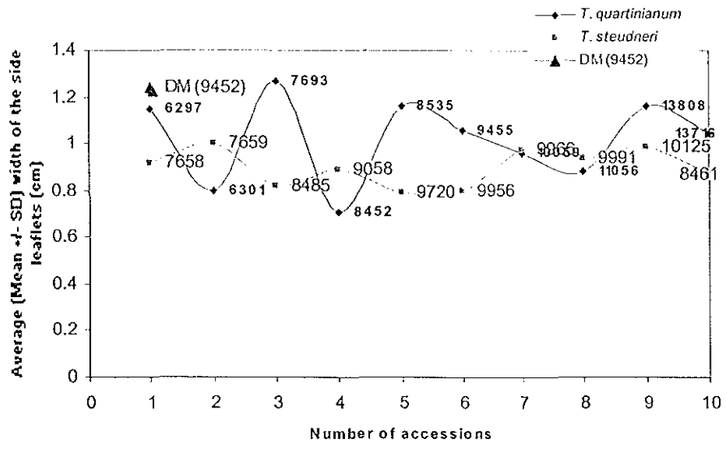


**Figure 6. Average (Mean ± SD) width of the side leaflets**

### 4.1.7. Leaflet length to width ratio of the terminal leaflet

*T. quartinianum* accessions

In this group of accessions, the highest leaflet length to width ratio (6.59 cm) was obtained for 10059, while the lowest (2.74 cm) was recorded for 8535. The average ratio for the species was 4.39 ± 1.99 cm. Accession 9455 showed the largest variability (4.83), where as accession 7693 showed the smallest variability (0.24). Appendix 1 and figure 13 summarize the results. One sample T- test performed showed that there was a significant difference (P= 0.006) among the accessions of *T. quartinianum* and the distinct morphotype at P< 0.05.
Results of test of homogeneity (P= 0.000) and ANOVA (P= 0.006) showed that the group variances were not equal and there was significant difference among the means, respectively.

## *T. steudneri* Accessions

The average leaflet length to width ratio of the species was 4.52 ± 0.94 cm. Accession 9720 had the largest (6.29 cm) mean of the species, whereas accession 9452 (the distinct morphotype) had the smallest (2.98 cm). Accession 10125 showed the smallest variability (0.18) while the largest variability (1.01) was observed in accession 8485 (Appendix 1 and Figure 14). One sample T- test witnessed the presence of significant difference (P= 0.000) between accessions of *T. steudneri* and the distinct morphotype at P< 0.05. There was homogeneity (P= 0.364) and significant difference (P= 0.000) among the group variances and averages respectively when test of homogeneity and ANOVA were performed at P< 0.05.

All accessions of the three taxa were combined and test of homogeneity of variances was calculated (P= 0.000) which showed that the variances were not homogenous both at P< 0.05. ANOVA was also computed on this combination and witnessed that there was significant difference (P= 0.000) among the means at P< 0.05. When test of homogeneity of variances was performed on the two species, it was found that there was homogeneity (P= 0.228) at P< 0.05. ANOVA witnessed that the two species had significant (P= 0.00) difference from each other (Appendix 2).

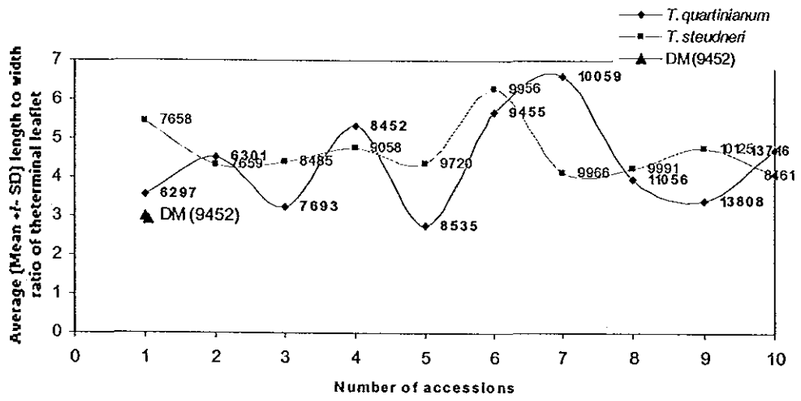


**Figure 7. Average (Mean ± SD) length to width ratio of the terminal leaflet**

### 4.1.8. Length to width ratio of the side leaflets

*T. quartinianum* accessions

The average leaflet length to width ratio of the species was 4.45 ± 1.17 cm. The highest mean leaflet length was 6.17 ± 0.73 cm and was recorded for 8452. The lowest average (3.08 ± 0.24 cm), on the other hand, was obtained for 8535. High variability (1.11) was observed in accession 13716, and low variability (0.24), by 8535 (Appendix 1 and Figure 15). A significant difference (P= 0.002) was observed among the accessions of *T. quartinianum* and the distinct morphotype, when one sample T- test was done at P< 0.05. ANOVA and test of homogeneity were also performed on the accessions of this species. ANOVA (P= 0.000) witnessed that there were significant differences among the average values at P< 0.05. Test of homogeneity (P= 0.007) indicated that the group variances were not homogenous at P< 0.05.

*T. steudneri* Accessions

Accession 9720 scored the highest (5.70 ± 1.12 cm) leaflet length to width ratio, whereas the distinct morphotype scored the least (3.06 ± 0.10 cm). The average leaflet length to width ratio of the species was 4.83 ± 0.86 cm. The largest variability (1.12) was observed in accession 9720; the smallest (0.10), in the distinct morphotype (Appendix 1 and figure 16). One sample T- test showed a significant (P= 0.000) difference among the accessions of *T. steudneri* and the distinct morphotype at P< 0.05. A significant difference (P= 0.024) was observed among the means as was shown by ANOVA. Test of homogeneity of variances showed that the group variances were not homogenous (P= 0.000) at P< 0.05.

Test of homogeneity of variances and ANOVA were calculated for the three taxa. The variances were found out to be non-homogenous (P= 0.000) by the test at P< 0.05. One way

ANOVA showed that there was a significant (P= 0.000) difference among the accessions of the three taxa at P< 0.05. Test of homogeneity of variances and ANOVA performed on the two species showed that the group variances were not homogenous (P= 0.029) and that there was no significant difference (P= 0.687) between them (Appendix 2).

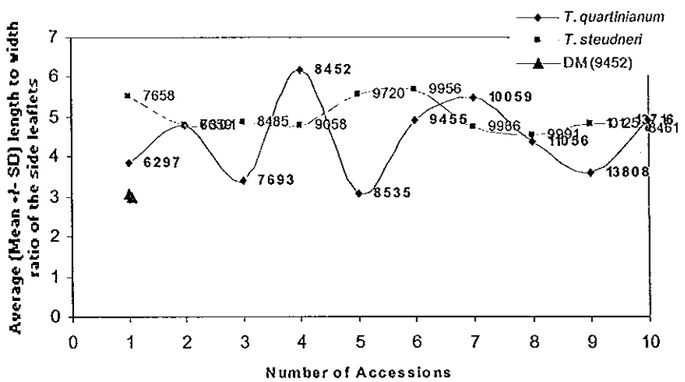


**Figure 8. Average (Mean ± SD) length to width ratio of the side leaflets**

### 4.1.9. Number of pods per inflorescence

*T. quartinianum* accessions

The average number of pods per inflorescence of the species was found to be 13.1 ± 8.4. The largest (26.6) and lowest (5.71) averages were observed in accessions 11056 and 13716, respectively. Accession 11056 showed the largest variability (6.44), while accessions 6301 and 8535 showed the smallest (0.000) variability (Appendix 1 and Figure 17). A significant difference (P= 0.000) was observed among the accessions when one-way ANOVA was computed at P < 0.05 and P< 0.01. Test of homogeneity also showed that the group was not homogenous (P= 0.000) at P < 0.05 and P< 0.01. Results of one sample T- test indicated (P= 0.051) that there was no significant difference between the mean values of the distinct morphotype and *T. quartinianum* accessions both at P< 0.05.

## *T. steudneri* Accessions

Accession 9058 scored the highest (14.3) average mean number of pods per inflorescence, while 7659 scored the smallest (7.1). The average for the species was 10.8 ± 3.84. The highest (3.98) and the smallest (0.21) variability was observed for accessions 8485 and 7659 respectively (Appendix 1 and figure 18). ANOVA and test of homogeneity were calculated at $P< 0.05$. ANOVA showed ($P= 0.000$) that there was a significant difference among the group means considered. Test of homogeneity was also computed at $P < 0.05$ and $P< 0.01$ and showed that the group variances were homogenous ($P = 0.173$). A significant difference ($P= 0.000$) was observed when one sample T- test was calculated between the mean value of the distinct morphotype and accessions of this species at $P< 0.05$.

Test of homogeneity of variances, performed on the total accessions, showed that the variances were not homogenous ($P = 0.00$) at $P< 0.05$. One-way ANOVA was also computed and showed that there was a significant difference ($P = 0.000$) among the means at $P < 0.05$ and $P< 0.01$. The two species were compared using ANOVA at $P< 0.05$ and were shown not to differ significantly ($P= 0.115$) (Appendix 2).

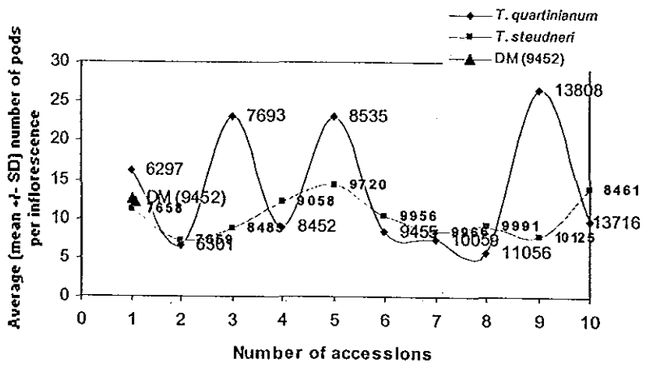


**Figure 9. Average (Mean ± SD) number of pods per inflorescence**

### 4.1.10. Number of seeds per pod

*T. quartinianum* accessions

The average number of seeds per pod for the species was 2.63 ± 1.25. The largest and smallest averages were 4.4 (for accession 9455) and 1.86 (for accession 7693), respectively. The largest variability (1.39) was observed for accession 10059, while the smallest (0.00) was observed for accessions 6301 and 8535 (Appendix 1 and figure 19). Statistical analysis was carried out by one way ANOVA at P< 0.05 and showed that there was no significant (P= 0. 079) difference among the means of the *T. quartinianum* accessions. Test of homogeneity of variances was also used at P< 0.05 and showed that the variances were homogenous (P = 0.302). When one sample T- test was conducted between the mean values of the accessions of this species and that of the distinct morphotype, no significant difference (P= 0.06) was observed both at P< 0.05.

*T. steudneri* Accessions

Accession 7658 showed the lowest (2.07) average number of seeds per pod, while 8461 showed the highest (2.64). The average number of seeds for the species was 2.26 ± 0.192. The largest variability (1.09) was observed for 9956. Accession 7659 exhibited the smallest variability (0.069) (Appendix 1 and figure 20). Statistical analysis was performed using ANOVA and test of homogeneity of variances. One way ANOVA showed that there was significant difference (P= 0.055) among the means of *T. steudneri* accessions at P<0.05 and P< 0.05. Test of homogeneity revealed that the group variances were equal (P= 0.07) (Appendix). A significant difference (P= 0.000) was observed between *T. steudneri* accessions and the distinct morphotype by one sampled T- test at P< 0.05.

Test of homogeneity of variances and ANOVA were computed at P< 0.05. Test of homogeneity showed that the variances were not homogenous (P= 0.000). ANOVA showed the presence of significant (P= 0.00) difference among the means of all accessions. A significant difference (P= 0.416) was not observed between the two species when ANOVA was computed at P< 0.05 (Appendix 2).

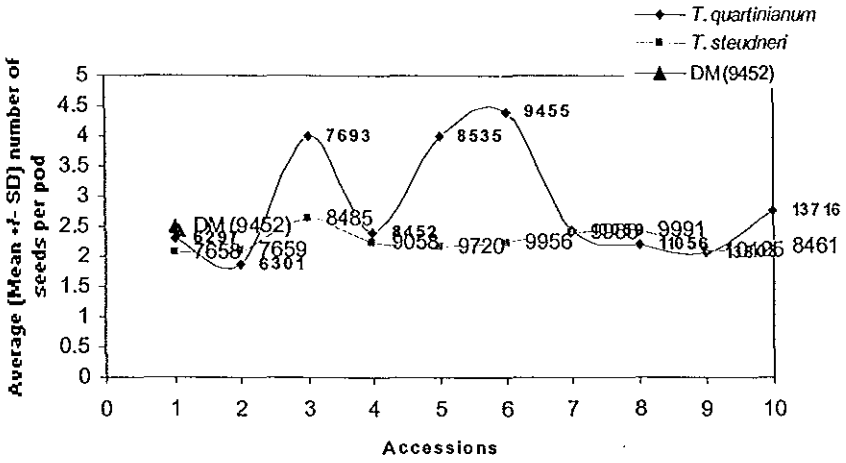


**Figure 10. Average (Mean ± SD) number of seeds per pod**

## 4.1.11. Length of the corolla

### *T. quartinianum* accessions

The average length of the corolla was found to be 1.51 ± 0.10 cm for the species. Accession 7693 scored the highest mean value (1.66 cm), while 11056 scored the smallest (1.44 cm). The highest variability (0.20) was observed in accession 8452. Accession 13808 showed the lowest variability (0.04) of the group (Appendix 2 and figure 21). One way ANOVA performed and indicated that there was a significant (P= 0.000) difference among the mean values of the accessions of *T. quartinianum.* The group was proved to be homogenous (P= 0.590) by test of homogeneity of variances at P< 0.05. One sample T- test was calculated to see if there exists a significant difference between the accessions of *T. quartinianum* and the

distinct morphotype. The test showed that there was a significant (P= 0.000) difference at P< 0.05.

## *T. steudneri* Accessions

The average length of the corolla for this species was found to be 0.84 ± 0.076 cm. The least variability (0.000) was observed for accession 8485, while the largest (0.07) was observed in accessions 8461, 9058, 9720, 10125 and 9966. Accession 9991 had the largest average (0.92 cm) length, whereas 7658 had the smallest (0.76 cm) (Appendix 1 and Figure 22). The results of one way ANOVA indicated that there was significant difference (P= 0.016) among the means of the *T. steudneri* accessions at P< 0.05. Test of homogeneity, on the other hand, showed that the group variances were not homogenous (P =0.039) at P< 0.05. One sample T-test witnessed that there was significant (P= 0.000) difference between the average values of accessions of this species and the distinct morphotype at P< 0.05.

Test of homogeneity of variances performed on the combined accessions showed that there was no homogeneity (P= 0.000) among the variances both at P< 0.05. ANOVA was calculated for this combination and showed that there was a significant (P= 0.000) difference among the means at P< 0.05 (Figure 23 and Appendices 1). When ANOVA was computed between the two species, a significant difference (P= 0.000) was obtained.

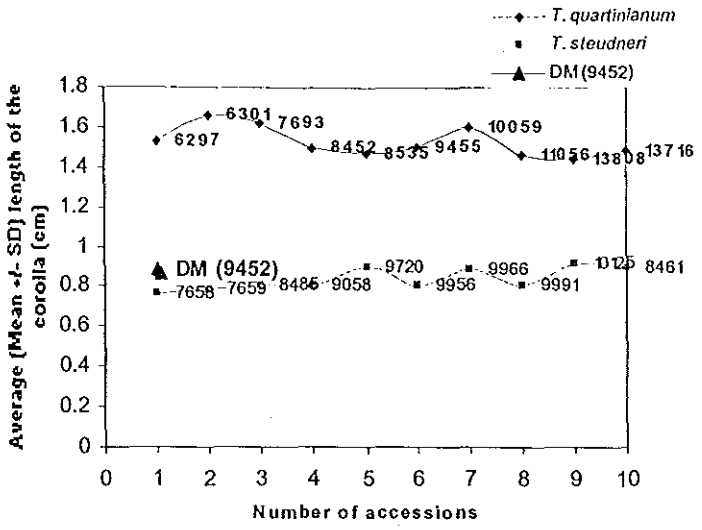


**Figure 11. Average (Mean ± SD) length of the corolla of all accessions.**

## 4.1.12. Length of the calyx-lobe

### *T. quartinianum* accessions

Accessions 8452 scored the highest (0.92 cm) average calyx –lobe length. The smallest average length (0.80 cm) was scored by 13716. The average length of the calyx-lobe for the species was found to be 0.86 ± 0.05 cm. The highest variability (0.07) was observed in accession 13716, whereas the lowest variability (0.000), in accession 11056 (Appendix 1 and Figure 24). One way ANOVA indicated that there was significant (0.005) difference at P< 0.05. Test of homogeneity of variances showed that the group variances were unequal (P= 0.015) at P< 0.05. One sample T- test was performed for accessions of *T. quartinianum* and the distinct morphotype and showed that there was no significant (P= 0.051) difference between the accessions of this species and the distinct morphotype at P< 0.05.

## *T. steudneri* Accessions

The average length of the calyx-lobe of this species was 0.43 ± 0.12 cm. High variability (0.11) in the length of the calyx-lobe was observed in accession 8461. Accession 9058 showed the lowest (0.043) variability for this character. The largest average length (0.52 cm) was recorded for accession 9991. Accession 9058, on the contrary, showed the smallest (0.12 cm) calyx-length (Appendix 1 and figure 25). The group was shown to be non- homogenous (P= 0.000) by test of homogeneity of variances at P < 0.05 and P< 0.01. One way ANOVA was computed and showed that there was no significant difference (P= 0.07) among the mean values of *T. steudneri* accessions at P< 0.05. The distinct morphotype was proved to be significantly different (P= 0.000) from the *T. steudneri* accessions by one sample T- test at P< 0.05. ANOVA was computed between the two species that showed that there was significant (P= 0.000) difference at P< 0.05.

Test of homogeneity of variances and ANOVA were calculated for the total accessions. Test of homogeneity showed that there was not homogeneity (P= 0.000) at P< 0.05. ANOVA (at P< 0.05) showed that there existed a significant (P= 0.000) difference among the means of the combined accessions (Figure 26 and Appendix 2).

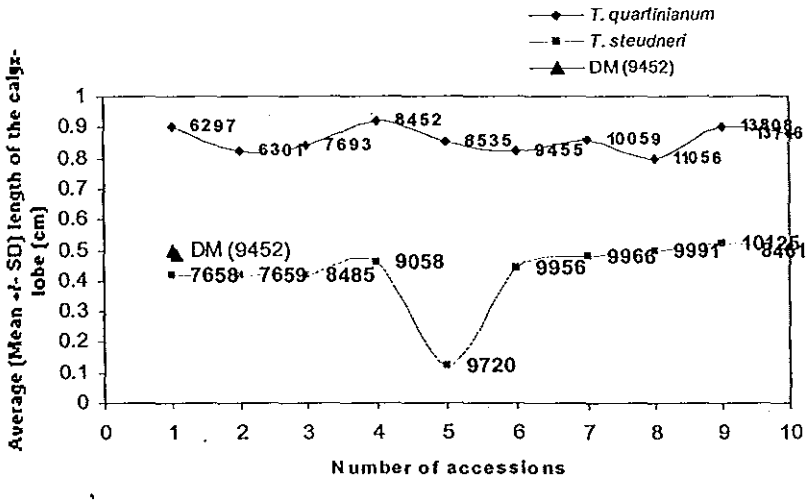


**Figure 12. Average (Mean ± SD) length of the calyx-lobe of all accessions.**

### 4.1.13. Leaf marks

The presence and absence of leaf marks was recorded as 0 (Presence) and 1 (Absence). The result was described in table 2. Among the accessions considered, 61.9 % had leaf marks while the rest had no leaf marks.

**Table 2. Presence (1) or Absence (0) of Leaf Marks**

| *T. quartinianum* Accessions | Leaf marks (0,1) | *T. steudneri* accessions | Leaf marks (0,1) |
|---|---|---|---|
| 6297 | 1 | 7658 | 0 |
| 7693 | 1 | 7659 | 1 |
| 6301 | 1 | 8461 | 0 |
| 8452 | 0 | 8485 | 1 |
| 8535 | 1 | 9058 | 1 |
| 9455 | 1 | 9720 | 0 |
| 10059 | 0 | 9956 | 1 |
| 13716 | 0 | 9966 | 1 |
| 11056 | 1 | 9991 | 1 |
| 13808 | 0 | 10125 | 1 |
| | | 9452 | 0 |

In *T. steudneri* accessions, 36 % of the accessions had leaf marks (64 % had no leaf marks, non- significant difference to the hypothesized test value (0.5); P= 0.549) while in *T. quartinianum* accessions, 60 % of the accessions had leaf marks (non- significant; P= 0.754).

Non-parametric test (Binomial test with test proportion = 0.5; 0.5 because the characters were assumed to have equal probability of occurrence in the population) was done and showed that 62 % of the total accessions had leaf marks while the remaining 38 % had no leaf marks. It is also observed that the observed leaf mark trend is consistent (P= 0.383) with the expected 0.5 (1:1) test proportion.

### 4.1.14. Direction of leaf serration

The direction of the leaf margin serration was also recorded. It fell into either of the following classes: forward (F) and perpendicular to the leaf margin (P). Leaf serrations directed forward were registered as (1), and those directed perpendicular to the margin, as (0) (Table 3). All accessions except *T. steudneri* (9966) had leaf serrations directed forward (4.8 %). In *T. steudneri* (9966), however, the serrations were perpendicular to the leaf margin. The distinct morphotype showed no difference to most of the accessions on the basis of this character. Its leaves had serrations directed to the forward direction.

**Table 3. Direction of Serration of the Leaf Margins.**

| *T. quartinianum* accessions | Leaf Serration Direction | *T. steudneri* accessions | Leaf Serration Direction |
|---|---|---|---|
| 6297 | 1 | 7658 | 1 |
| 7693 | 1 | 7659 | 1 |
| 6301 | 1 | 8461 | 1 |
| 8452 | 1 | 8485 | 1 |
| 8535 | 1 | 9058 | 1 |
| 9455 | 1 | 9720 | 1 |
| 10059 | 1 | 9956 | 1 |
| 13716 | 1 | 9966 | 0 |
| 11056 | 1 | 9991 | 1 |
| 13808 | 1 | 10125 | 1 |
| | | 9452 | 1 |

All (100 %) accessions of *T. quartinianum* had leaf serrations directed forward, while 91% of *T. steudneri* had this feature. The binomial test showed that there was a significant (P= 0.002, and P= 0.012) difference in the direction of leaf serration of *T. quartinianum* and *T. steudneri* accessions, respectively.

Non-parametric test (Binomial test with test proportion = 0.5) showed that 95 % of the total accessions had leaf serrations directed forward. Only 5% had serrations directed perpendicular to the leaf margin. Binomial test done for all the accessions showed a highly significant (P =0.000) difference from the hypothesized test proportion (0.5).

### 4.1.15. Nature of the stipule

The nature of the stipule was observed and recorded as (0) for entire margins and (1) for serrated margins. All accessions (95.2 %) of *T. quartinianum* and *T. steudneri* had stipules with smooth margin. The margins of the stipules were different only for the distinct morphotype (4.8 %). The stipule margins of the distinct morphotype were serrated and distinct when compared to the rest of the accessions (Table 4).

**Table 4. Nature of the stipule margin**

| *T. quartinianum* accessions | Stipule Nature | *T. steudneri* accessions | Stipule Nature |
|---|---|---|---|
| 6297 | 0 | 7658 | 0 |
| 7693 | 0 | 7659 | 0 |
| 6301 | 0 | 8461 | 0 |
| 8452 | 0 | 8485 | 0 |
| 8535 | 0 | 9058 | 0 |
| 9455 | 0 | 9720 | 0 |
| 10059 | 0 | 9956 | 0 |
| 13716 | 0 | 9966 | 0 |
| 11056 | 0 | 9991 | 0 |
| 13808 | 0 | 10125 | 0 |
| | | 9452 | 1 |

Binomial test with a test proportion of 0.5 was performed and showed that there was a significant difference (P=0.002, 0.012 and 0.000 for *T. quartinianum*, *T. steudneri* and total accessions respectively) from the hypothesized value for both species.

## 4.2. Cluster Analysis

Cluster Analysis was made following the hierarchical cluster analysis procedure (SPSS, Version 10.0, 1999). The morphological characters were used to undergo the cluster analysis. Dendrograms were constructed for the combination of all the accessions of *T. quartinianum*, *T. steudneri* and the distinct morphotype. This was done so as to see the relative relation of the distinct morphotype to the accessions of both species (Figure 13).

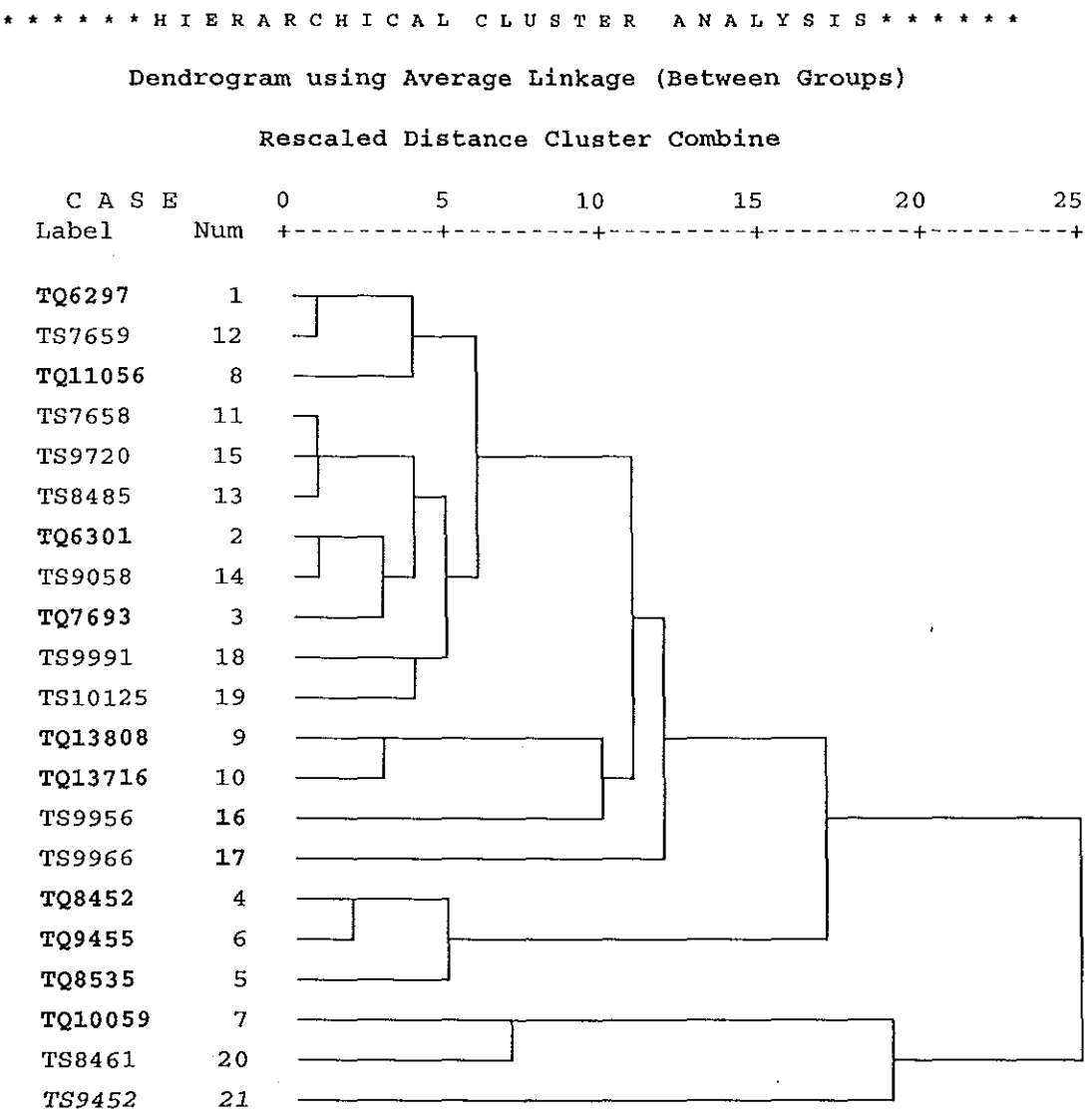


**Figure 13. Dendrogram constructed (on a rescaled scale) based on the combination of all the characteristics of all the accessions.**

Euclidean distance was calculated for all the twenty one accessions and the distance (dissimilarity coefficient) was used to construct the dendrogram. The dendrogram hence generated resulted in grouping of all the accessions into nine clusters. The clusters generated (on a rescaled scale) and the accessions in them are summarized in Table 5.

**Table 5. The clusters generated and the accessions included in the clusters by hierarchical cluster analysis procedure.**

| Clusters | Accession Numbers |
|---|---|
| I | **6297, 7659, 11056** |
| II | **7658, 9720, 8485** |
| III | **6301, 9058, 7693** |
| IV | **9991, 10125** |
| V | **13808, 13716** |
| VI | 9956 |
| VII | 9966 |
| VIII | **8452, 9455, 8535** |
| **IX** | **10059, 8461** |
| **X** | 9452 |

The distinct morphotype was found clustered close to accessions *T. quartinianum* (10059) and *T. steudneri* (8461). The Euclidean distance between the distinct morphotype and accession 10059 was 3.86. The distance (dissimilarity index) was 5.85 between the distinct morphotype and accession 8461 (Appendix ). The distinct morphotype, hence, was relatively closer to accessions of *T. quartinianum* (10059) than all other accessions. Accessions 10059 and 8461 were found in the same subgroup where 9452 belonged. Accession 9452 was an outlier from this subgroup. The hierarchical cluster analysis generated some other outliers which stood alone by themselves. These include *T. steudneri* accessions 9956 (Cluster VI) and 9966 (Cluster VII).

When the three accessions (in cluster IX and X) were compared with each other on the basis of the place of collection, it was found that accessions 10059 and the distinct morphotype were collected from Welega and Metekel, respectively while accession 8461 was collected

from Kefa (Jima). The relatively higher closeness of accessions 9452 and 10059 may be explained by the closeness of the sites where they are collected.

## Discriminant Analysis

Discriminant analysis was performed to see which of the characters were discriminated the three taxa. The Eigen values showed that two functions were generated and the first function extracted 97.6 % of the variance while the second extracted 2.4% of the total variance. The three taxa were discriminated on the basis of the discriminating characters. The prior probabilities were calculated for each of the groups and showed that the group membership probability was 0.50 for *T. quartinianum;* 0.44 for *T. steudneri* and 0.06 for the distinct morphotype. The percentage of the groups that were in the initial group and fitting into the final member ships was calculated and showed that there was a 97.14 % agreement (Appendix 6). The distinct morphotype was closer to *T. steudneri* accessions that to *T. quartiniaum* accessions (Figure 14).

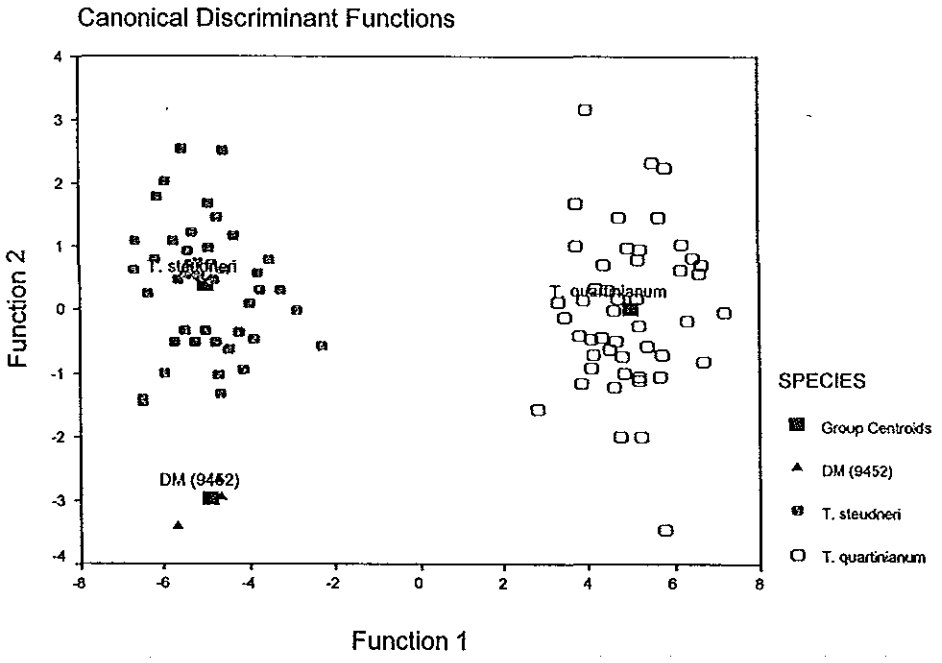


**Figure 14. The three groups generated by discriminant analysis.**

## 4.3. Principal Component Analysis

Principal component analysis (PCA) was computed and generated seven components. KMO and Bartlett's test were performed prior to calculating PCA. The results (Table 6) of the KMO and Bartlett's tests testified that it was advisable to perform the PCA test. Those components that had Eiegen values greater that 1 were retained in this analysis.

**Table 6. Results of the KMO and Bartlett's Test**

| | | |
|---|---|---|
| Kaiser-Meyer-Olkin Measure of Sampling Adequacy. | | .384 |
| Bartlett's Test of Sphericity | Approx. Chi-Square | 349.459 |
| | df | 105 |
| | Sig. | .000 |

Eigen values were calculated (Table 7) and showed that component 1 extracted 17.8 % (cumulative of 17.8 %) of the total variance. Components 2 and 3 extracted 13.3 % (cumulative of 31.0 %) and 11.1 % (cumulative of 42.2 %) of the total variance, respectively. The seven components, as a whole, extracted 75.1 % of the total variance. Out of the fifteen morphological characters used (i.e. fifteen dimensions), seven components were generated. The correlation of the individual characters to the components generated was shown by the rotated space component matrix.

**Table 7. Eigen values before and after extraction of the Components.**

| Component | Initial Eigenvalues | | | Extraction Sums of Squared Loadings | | |
|---|---|---|---|---|---|---|
| | Total | % of Variance | Cumulative % | Total | % of Variance | Cumulative % |
| 1 | 2.666 | 17.775 | 17.775 | 2.666 | 17.775 | 17.775 |
| 2 | 1.989 | 13.260 | 31.035 | 1.989 | 13.260 | 31.035 |
| 3 | 1.678 | 11.186 | 42.221 | 1.678 | 11.186 | 42.221 |
| 4 | 1.433 | 9.553 | 51.774 | 1.433 | 9.553 | 51.774 |
| 5 | 1.402 | 9.347 | 61.121 | 1.402 | 9.347 | 61.121 |
| 6 | 1.074 | 7.159 | 68.280 | 1.074 | 7.159 | 68.280 |
| 7 | 1.027 | 6.846 | 75.126 | 1.027 | 6.846 | 75.126 |
| 8 | .877 | 5.847 | 80.973 | | | |
| 9 | .775 | 5.170 | 86.143 | | | |
| 10 | .733 | 4.886 | 91.029 | | | |
| 11 | .597 | 3.981 | 95.010 | | | |
| 12 | .540 | 3.599 | 98.609 | | | |
| 13 | .104 | .694 | 99.303 | | | |
| 14 | 8.224E-02 | .548 | 99.851 | | | |
| 15 | 2.234E-02 | .149 | 100.000 | | | |

Extraction Method: Principal Component Analysis.

Varimax rotation was adopted and showed that component 1 was loaded high with the total length of the corolla and the calyx-lobe. Leaflet length to width ratio of the side leaflets and width of the side leaflets are the characters that showed high correlations with component 2. Component 3, on the other hand, showed high correlations with leaflet length to width ratio of the terminal leaflet and width of the terminal leaflet. Size of the flowers for the pollen to shed and for the stigma to be receptive showed higher correlations with component 4. Component 5 showed high component loadings with number of pods per inflorescence and total length of the side and terminal leaflets. Plant height was observed to correlate with component 6, while number of seeds per pod with and without bagging showed high correlations with component 7 (Table 8).

**Table 8 Rotated Component Matrix for the Characters used**

| | Component | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| LCLTOT | .954 | -2.48E-02 | -6.84E-02 | -4.01E-04 | 7.648E-02 | 3.618E-02 | -5.16E-02 |
| LOCTOT | .907 | 1.621E-02 | -.130 | -5.56E-02 | -8.95E-03 | -7.28E-03 | -9.50E-02 |
| HSW | .433 | .228 | .104 | .334 | .300 | .352 | -.123 |
| RATTOT | -9.24E-02 | .957 | 4.188E-02 | -7.62E-02 | .167 | -2.55E-02 | 9.265E-02 |
| WSLTOT | -.121 | -.869 | -6.49E-02 | -7.85E-02 | .225 | 9.703E-03 | 4.739E-02 |
| RATTOT1 | -2.21E-03 | 3.050E-02 | .956 | -5.77E-02 | 5.584E-02 | .163 | -2.31E-02 |
| WFLTOT | .205 | -7.73E-02 | -.864 | 2.991E-02 | .219 | .260 | -5.08E-02 |
| SSR | -7.13E-02 | -6.91E-02 | -6.03E-02 | .803 | 9.282E-02 | -8.81E-02 | -1.99E-02 |
| SPS | 5.231E-02 | 4.271E-02 | -2.05E-02 | .782 | -3.60E-02 | 3.987E-02 | 3.724E-02 |
| NPITOT | -.191 | .156 | .141 | -.147 | -.701 | 5.481E-02 | 2.140E-03 |
| LSLTOT | -.372 | .355 | -8.22E-02 | -.221 | .517 | -6.55E-02 | .225 |
| PHT | -5.41E-02 | 8.835E-02 | .103 | 3.914E-02 | .142 | -.836 | -4.10E-02 |
| LFLTOT | -3.59E-02 | 8.091E-02 | .158 | -3.26E-02 | .527 | .597 | -.116 |
| NSPTOT | -9.53E-02 | 3.990E-02 | -2.98E-03 | 4.611E-02 | -.157 | .128 | .778 |
| NSPBTOT | -6.04E-02 | 6.395E-03 | 1.963E-02 | -3.21E-02 | .172 | -.149 | .748 |

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

The varimax rotation was applied to come up with the rotated component plot that is shown in Figure 15. The characters in each of the components generated showed high correlations with each other and with the component. This is in agreement to the general expectations.

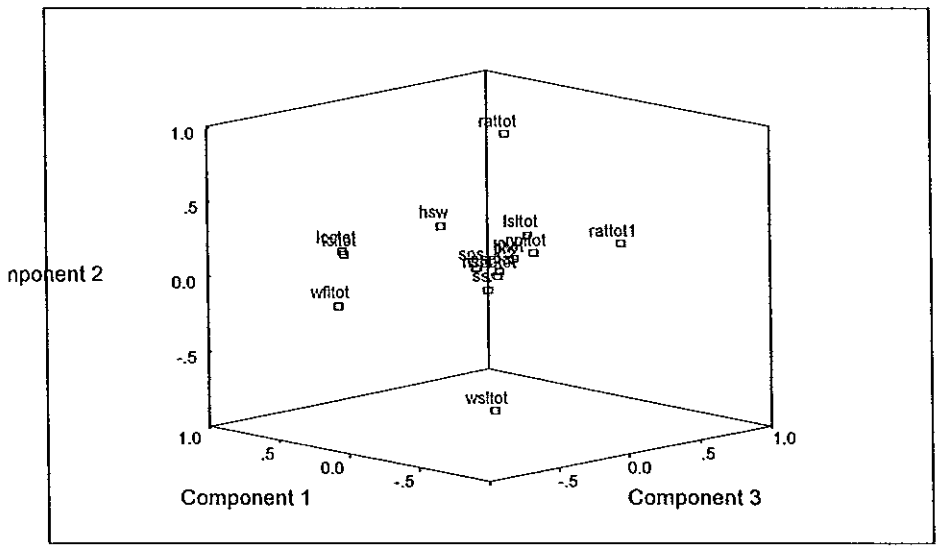


**Figure 15. Component plot of the characters on a rotated space**

The relationships among the accessions of the two species were observed using certain pairs of morphological characters. The accessions of each of the two species were separated on the basis of these characters. These characters are length of the calyx-lobe and the corolla and the sizes of the flower for pollen shedding and stigma receptivity. These can, hence, be used as key morphological characters to distinguish among the species (Figure 16).

Three groups were generated by using the first pairs of characters. These were the average length of the calyx-lobe and the corolla. The scatter plot for the combination of these characters showed that accessions of *T. quartinianum* were found at higher average values for length of the calyx and the corolla. The distinct morphotype, on the other hand, was found

together with *T. steudneri* accessions. This group was observed at lower average values than those accessions of the *T. quartinianum*. Accession 9720 (a *T. steudneri* accession) was found out of the ranges of the two groups.

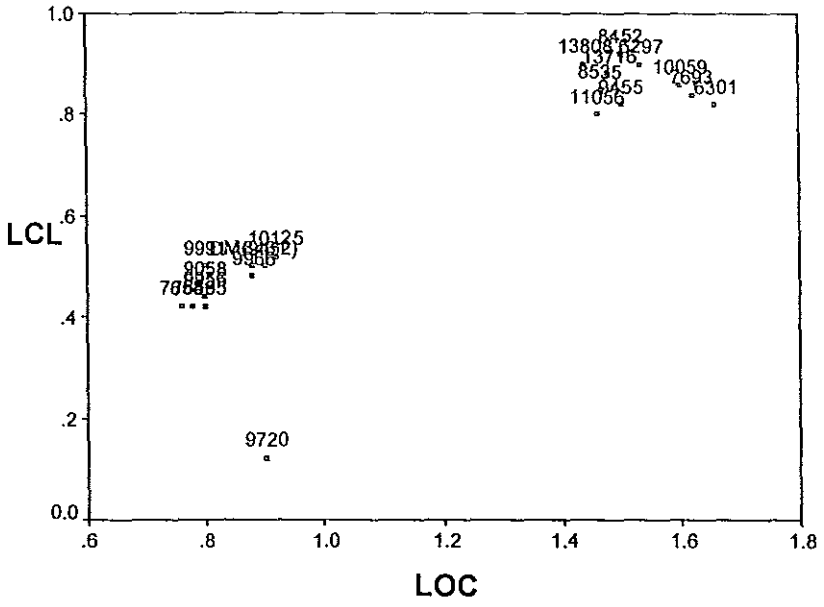


**Figure 16. Scatter plot showing the relationships among accessions of the two species constructed based on the length of the calyx-lobe and the corolla.**

On the basis of the other pairs of characters (i.e. sizes of the flowers for pollen shedding and stigma receptivity) three groups were generated. The first group included accessions of the distinct morphotype and seven accessions of *T. quartinianum*. The second group was rather heterogeneous with regard to the accessions in that it included all accessions of *T. steudneri* and two accessions (13716 and 7693) of *T. quartinianum*. The third group was relatively far from these groups and was represented by an accession of *T. quartinianum* (10059). These pairs of characters, again, can be used to cautiously classify the two species (Figure 17).

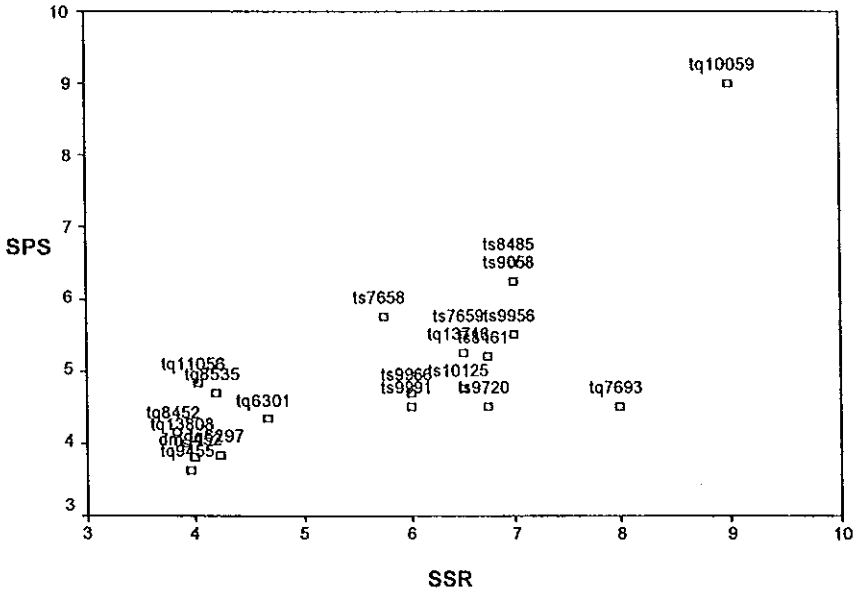


**Figure 17. Scatter plot showing the relationships among accessions of the two species constructed based on the length of the sizes of the flowers for the pollen to shed and the stigma to be receptive.**

## 4.4. Hybridization

### 4.4.1. Pollen shedding

*T. quartinianum* Accessions

The average size of the flower buds that was associated with pollen shedding was 4.66 ± 1.79 cm for this species. Accession 10059 shed the pollen at the largest mean flower size of the group (9.00 ±2.83 cm), while 9455 at the smallest (3.63 ± 0.78 cm) size. The largest variability (2.83) in the flower sizes associated with pollen shedding was observed in accession 10059, whereas the smallest (0.76) was observed in 6297 and 6301(Appendix 1 and Figure 18). There was no significant difference (P= 0.016) among the mean values of the distinct morphotype and the *T. quartinianum* accessions when one sample T- test was computed at P< 0.05. The results of ANOVA showed that there was not a significant difference (P= 0.225) among the mean values at P< 0.05. Test of homogeneity of variances was also performed and witnessed that the group variances were not homogenous (P= 0.005) at P< 0.05.

*T. steudneri* Accessions

The distinct morphotype shed its pollen at the smallest average bud size (3.80 ± 0.12 cm) of the species. It also exhibited the least variability (0.12) of the group. The highest average mean size (6.50 ± 1.80 cm) and variability (2.55) were observed for accessions 8485 and 8461, respectively. The average size for the species was found to be 5.13 ± 1.60 cm (Appendix 1 and Figure 18). One sample T-test showed the presence of significant (P= 0.000) difference among the means at P< 0.05. ANOVA (P= 0.05) witnessed that there was not significant difference among the average values at P< 0.05. Test of homogeneity (P= 0.000) showed that the group variances were not homogenous at P< 0.05.

Test of homogeneity of variances and ANOVA were calculated for all accessions. Test of homogeneity of variances showed the absence of homogeneity (P= 0.005) at P< 0.05. ANOVA witnessed that there was not significant difference (P= 0.670) among the means at P< 0.05. Test of homogeneity of variances and ANOVA were computed between the two species at P< 0.05. Test of homogeneity witnessed that the variances were homogenous (P= 0.907), while ANOVA showed (P= 0.074) that there was no significant difference between them.

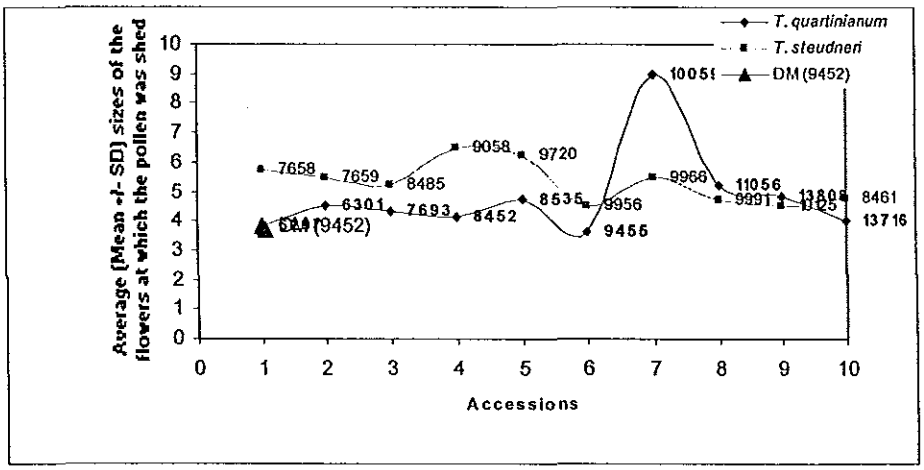


**Figure 18. Average sizes of the flowers of all accessions at which the pollen was shed.**

## 4.4.2. Stigma Receptivity

### *T. quartinianum* accessions

The size of the flower at which the stigma was receptive was measured. The average size for the species was 5.29 ± 2.06 cm. The maximum (9.00 ± 2.00 cm) and minimum (3.83± 0.29 cm) average sizes were scored by accessions 10059 and 8452, respectively. High variability (2.00) was recorded for accession 10059, while low variability (0.000) was observed in accession 7693 (Appendix 1 and Figure 19). One sample T- test showed that there was a significant difference (P= 0.002) among the mean size of the distinct morphotype and the

means of *T. quartinianum* accessions at P< 0.05. A significant (P= 0.000) difference was observed among the mean values when one way ANOVA was performed at P< 0.05. Test of homogeneity was also calculated and showed that there was no homogeneity (P= 0.000) at P< 0.05.

## *T. steudneri* Accessions

Accessions 8485 and 9058 scored the highest average mean size for stigma receptivity (7.0 ± 1.41 cm) while the distinct morphotype scored the least (4.00 ± 0.71 cm). The average for the species was 6.02 ± 1.30 cm. High variability (1.76) was observed in accession 9720. The lowest variability (0.00), on the other hand, was recorded for accessions 9956, 9966, and 9991 (Appendix 1 and Figure 19). Significant (P= 0.000) difference was observed among the means when one sample T- test was performed at P< 0.05. The group variances were found to be non- homogenous (P= 0.001) by test of homogeneity at P< 0.05. ANOVA witnessed that there was a significant (P= 0.01) difference among the means.

One way ANOVA showed that there was not significant difference (P= 0.000) among the means at P< 0.05. Test of homogeneity of variances indicated the variances were not homogenous (P = 0.000) at P< 0.05.

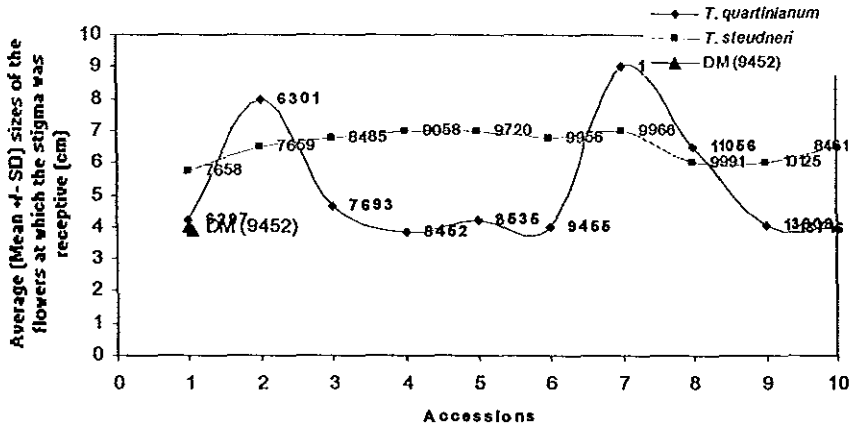


**Figure 19. Average sizes of the flowers of all accessions at which the stigma was receptive.**

Comparison of the sizes of the flowers at which the pollen was shed and the stigma was receptive was done to see if there exists any incompatibility. The average size of the flowers of *T. quartinianum* accessions at which the pollen was shed was 4.82 ± 1.55 cm while it was 5.24 ± 1.90 cm for the stigma to be receptive. The pollen grains of flowers of *T. steudneri* accessions were shed at 5.18 ± 0.81 cm while the stigma was receptive at average flower size of 6.29 ± 0.88 cm (Figure 20). ANOVA was computed at $P < 0.05$ and showed that there was a significant difference ($P = 0.003$) between the two species.

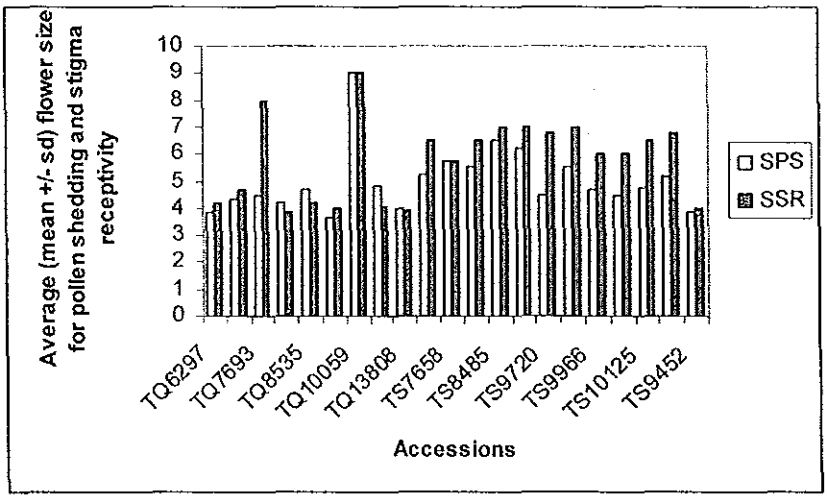


**Figure 20 Comparison of the size of the flowers at which the pollen was shed and the stigma was receptive.**

Pearson's correlation analysis was performed at $P < 0.01$ and showed that pollen shedding and stigma receptivity are strongly positively correlated ($P = 0.740$) and the correlation was significant ($P = 0.0000$) (Table 9).

**Table 9 Correlation coefficients for the sizes of the flowers at which the pollen was shed and the stigma was receptive.**

| | | SPS | SSR |
|---|---|---|---|
| SPS | Pearson Correlation | 1.000 | .740** |
| | Sig. (2-tailed) | . | .000 |
| | N | 21 | 21 |
| SSR | Pearson Correlation | .740** | 1.000 |
| | Sig. (2-tailed) | .000 | . |
| | N | 21 | 21 |

**. Correlation is significant at the 0.01 level

### 4.4.3. Pollen viability

Viability of the pollen grains was studied from the approximately first time the pollen was shed. There was a general decrease in pollen grain viability with time. The maximum and minimum numbers of viable grains were 28 and 15 (for *T. steudneri*; 9452), 35 and12 (for *T. steudneri*; 7659), 26 and 6 (for *T. steudneri*; 9720), 32 and 7 (for *T. steudneri*; 7658), 38 and 25 (for *T. steudneri*; 9991), 23 and 5 (for *T. quartinianum*; 6301), 33 and 12 (for *T. quartinianum*; 8535) and 26 and 16 (for *T. quartinianum*; 7693). The percent decrease of pollen viability was 46.4 % (for *T. steudneri*; 9452), 65.7 % (for *T. steudneri*; 7659), 76.9 % (for *T. steudneri*; 9720), 34.2 % (for *T. steudneri*; 9991), 78.3 % (for *T. quartinianum*; 6301), 63.6 % (for *T. quartinianum*; 8535), and 38.5 % (for *T. quartinianum*; 7693). *T. steudneri* (9991) showed an unpredictable trend in pollen viability in that a sudden increase was observed in the late hours of the observation. The times at which the pollen grains were taken for the study were: 0 hour (8: 30 am); 1 hour (12:00 am); 2 hours (2: 00 pm) and 3 hours (4: 00 pm) (Figure 21).

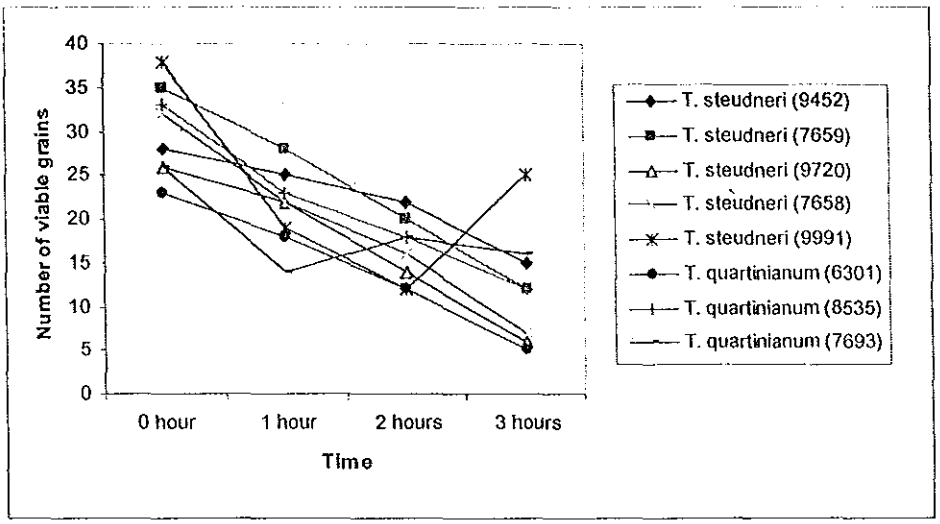


**Figure 21. Number of viable pollen grains with time.**

## 4.4.4. Pollen tube growth

Pollen tube growth was assessed in several species combinations involving a number of accessions. A number of intra- and interspecific crosses were examined in relation to pollen tube growth. The pattern of growth of the pollen tubes was recorded as 0 and 1; 1 representing "Pollen tube growth" and 0 "no pollen tube growth" (Table 10).

**Table 10. Pollen tube growth in intra- and interspecific crosses.**

| Crosses Made | | Nature of Crossing | Pollen-tube Growth |
|---|---|---|---|
| **Male Parent** | **Female Parent** | | |
| *T. steudneri (7659)* | *T. steudneri (7659)* | Self | 1 |
| *T. steudneri (7659)* | *T. steudneri (9452)* | Cross | 0 |
| *T. steudneri (9452)* | *T. steudneri (7659)* | Cross | 0 |
| *T. quartinianum (8535)* | *T. quartinianum (8535)* | Self | 1 |
| *T. quartinianum (8535)* | *T. steudneri (7659)* | Cross | 0 |
| *T. steudneri (7659)* | *T. quartinianum (8535)* | Cross | 1 |
| *T. steudneri (9452)* | *T. steudneri (9452)* | Self | 1 |
| *T. quartinianum (8535)* | *T. steudneri (9452)* | Cross | 1 |

In those crosses that had pollen tube growth, the callose in the pollen tubes fluoresced bright yellow with the background tissue fluorescing pale gray. The extent of the pollen tube growth varied among the accessions. Intraspecific crosses had better pollen tube growth than interspecific crosses. In most interspecific crosses, there was no growth of the pollen tubes in the style and no fluorescing callose tissue was observed. Appreciable levels of pollen tube growth were observed, however, in some interspecific crosses. Descriptive statistics showed that 33.3 % of the total crosses didn't show pollen tube growth while 66.7% showed pollen tube growth.

### 4.4.5. The Bagging Experiment

*T. quartinianum* accessions

The average number of seeds per pod obtained for the selfing, done with bagging, was 2.42 ± 1.96. The largest average (4.92) was recorded for 9455, while the lowest (0.00) was observed for accessions 7693, 6301, 8452 and 8535, in which case no seeds were produced. High variability (1.45) was observed in accession 10059. Accessions 7693, 6301, 8452 and 8535 exhibited the lowest variability (0.00) of the group (Appendix 1 and Figure 22). One way ANOVA was performed among the means of the accessions. The result of the ANOVA test showed that there was significant difference ($P = 0.00$) among the accessions at $P < 0.05$. Test of homogeneity was also calculated and showed that the group variances were not equal ($P = 0.00$) at $P < 0.05$.

## *T. steudneri* Accessions

Accession 9058 had the maximum average (2.77) number of seeds of the group. The minimum average (2.00) was scored by the distinct morphotype accession 9452. The group average was 2.31 ± 0.98. Accession 8461 showed the highest variability (1.14), while the distinct morphotype showed the smallest (0.00) variability (Appendix 1 and Figure 22). Results of one way ANOVA indicated that there was significant difference (P= 0.000) among the accessions at P< 0.05. The groups were observed to have unequal variances (P= 0.00), as shown by test of homogeneity of variances at P< 0.05.

Test of homogeneity of variances indicated that there was no homogeneity (P= 0.000) among the variances both at P< 0.05. One way ANOVA was also calculated which showed that there was a significant difference (P= 0.000) among the means of the combined accessions at P< 0.05. Test of homogeneity of variances and ANOVA were also computed on the two species and showed that the variances were not homogenous (P= 0.000) and that the two species were significantly different (P= 0.000) from each other at P< 0.05.

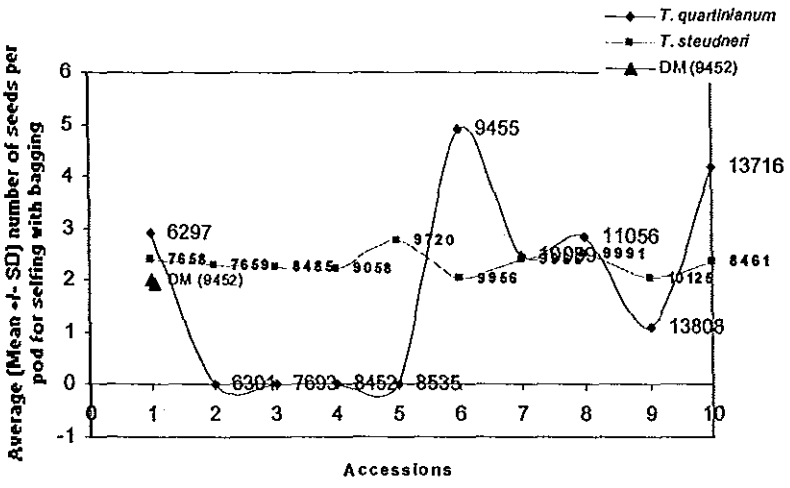


**Figure 22. Average (Mean ± SD) number of seeds per pod for selfing with bagging**

Paired samples T- test was performed to see if there existed differences in the number of seeds formed by selfing with- and without bagging. Paired samples T- test witnessed that there was not a significant (P= 0.417) difference among the means of the two experimental groups at P< 0.05 (Table 11).

**Table 11 Paired T-test for the Number of Seeds per Pod obtained by Selfing with- and without Bagging.**

| | Paired Differences | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|
| | Mean | Std. Deviation | Std. Error Mean | | | |
| Pair 1 NSP - NSPB | .4476 | 1.4965 | .3266 | 1.371 | 20 | .186 |

## 4.4.6. Interspecific hybridization and Seed formation

Intra- and interspecific hybridizations were performed among certain accessions of the two species and the distinct morphotype. Seeds were formed in some of the crosses, but not in others. Seed formation was recorded as 0 and 1; "1" for cases of seed formation and "0" for no seed formation. The result is summarized in Table 12.

**Table 12 Interspecific Hybridization and Seed formation in the crosses**

| Male Parent | Female Parent | Number of | | Seed Formation |
|---|---|---|---|---|
| *T. quartinianum* Accessions | *T. steudneri* Accessions | Male Flowers Used | Female Flowers used | |
| 11056 | 9058 | 5 | 5 | 1 |
| 8535 | 7658 | 5 | 5 | 1 |
| 8535 | 10125 | 2 | 2 | 1 |
| 6301 | 9966 | 5 | 5 | 1 |
| 10059 | 7659 | 3 | 3 | 1 |
| 13716 | 7658 | 5 | 5 | 1 |
| 13808 | 9058 | 3 | 3 | 0 |
| 6301 | 10125 | 5 | 5 | 0 |

Results of the crossing experiment showed that among the crosses made, 75% formed seeds while 25 % didn't form viable seeds. This showed that a significant level of compatibility exists among the accessions (Table 13).

**Table 13 Percentage of the crosses that resulted in seed formation (1) or not (0).**

| | | Frequency | Percent | Valid Percent | Cumulative Percent |
|---|---|---|---|---|---|
| Valid | no seed formation | 2 | 22.2 | 25.0 | 25.0 |
| | seed formation | 6 | 66.7 | 75.0 | 100.0 |
| | Total | 8 | 88.9 | 100.0 | |
| Missing | System | 1 | 11.1 | | |
| Total | | 9 | 100.0 | | |

## 4.5. Chromosome Numbers

The chromosome numbers of some accessions of the two species and the distinct morphotype were studied. The diploid chromosome number of both *T. quartinianum* and *T. steudneri* was found to be 2n = 16. This number was also observed for the distinct morphotype. Figures 23 and 24 show the chromosomes of typical accessions observed.

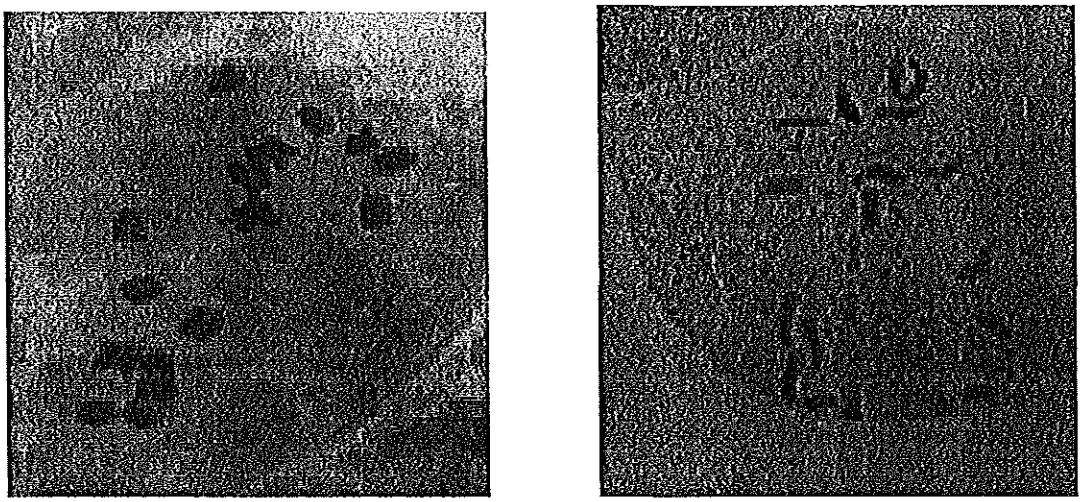

**Figure 23. Chromosomes (2n =16) of a typical *T. quartinianum* accession.**

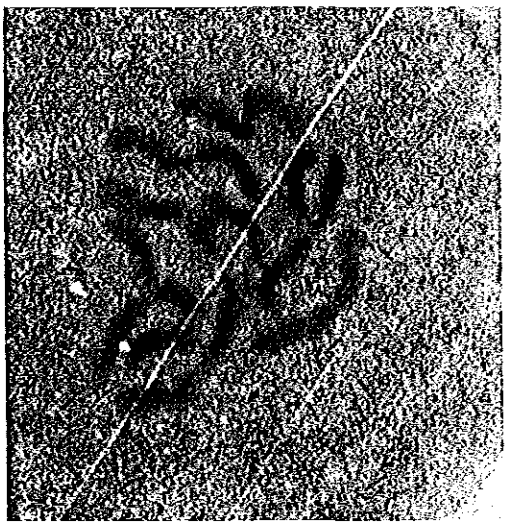

**Figure 24. Chromosomes (2n = 16) of the distinct morphotype (*T. steudneri*, 9452).**

# 5. DISCUSSIONS

## 5.1. Morphology

ANOVA was computed among the accessions and showed that there were significant difference among the species for all the characters except for the length of the terminal leaflet. There was also significant difference among accessions for all the characters. When ANOVA was computed, on the other hand, for the replications, there was no significant difference among them for most characters. Width of the side leaflets, width of the terminal leaflets and size of the flowers at which the pollen was shed were the characters that showed significant difference among the accessions. This analysis witnessed that the two species were distinct from each other on the basis of most of the characters. The distinct morphotype was compared to the two species based on some morphological characters and the level at which it showed significant difference, when it showed, was computed. Generally, it exhibited a unique array of morphological characters relative to accessions of the two species. The various statistical analyses revealed the level of difference with respect to each character and accession. These differences can be viewed from the point of view of the two species. The distinct morphotype showed highly significant difference with the accessions of both species on most characters. In most cases, the distinct morphotype stood out of the ranges defined by the two species. Homogenous subsets were generated for each of the characters and the species were grouped into these subsets based on their proximities. In some of the subsets, *T. quartinianum* and *T. steudneri* were in the same subset with the distinct morphotype being grouped in a different subset. In other cases, each of these species was found grouped with the distinct morphotype, with the distinct morphotype serving as an intermediate species (Appendix 5). The results of the discriminant analysis showed that on the basis of the factors generated, the three taxa were

clearly separated from each other and that certain characters were able to discriminate the three taxa.

Direction of leaf serration and nature of the stipule also showed highly significant differences among accessions. The distinct morphotype was characterized by serrated stipules while all the rest had smooth ones. Binomial test revealed that there was a significant difference among the accessions in relation to this trait. This character can, hence, be used as a key morphological characteristic of the distinct morphotype because it is found only in the distinct morphotype. Direction of leaf serration also showed significant difference among the accessions. Only a *T. steudneri* accession (9966), had leaves with serrations perpendicular to the leaf margin. All the rest had leaf serrations directed forward and binomial test showed significant difference with this regard. The presence or absence of leaf mark showed no significant difference both among accessions of the same and different species. Akundabweni *et al.*, (1991) used similar morphological characters to quantify the level of variation in *T. tembense.*

Using the morphological characters cluster analysis was computed. The cluster analysis (and the dendrogram constructed from the hierarchical cluster analysis) showed the relative relation of the distinct morphotype to accessions of *T. quartinianum* and *T. steudneri* and the relatedness of each of these accessions to each other. The distinct morphotype was observed to be relatively closer to accessions of *T. quartinianum* than *T. steudneri.* In fact, it was found in the same branch with the *T. quartinianum* accession, 10059. Some accessions (e.g. *T. steudneri* 9956 and 9966) were also observed to diverge away from the main body by this clustering process. When the two species were assessed in terms of the dendrogram constructed, they were found mixed to each other. The dendrogram showed that the two

species can not be distinguished from each other on the basis of the overall morphological characters used. Hence it was necessary to look for key characters which can be used to classify the taxa. Average length of the corolla and calyx-lobe and the sizes of the flowers at which the pollen was shed and the stigma was receptive were used in this line because they were to roughly classify the species. These characters nearly resulted in classifying the accessions into each of the species. Some accessions, however, were observed not to fit into the groups.

## 5.2. Hybridization

Two preconditions need to be fulfilled for successful hybridization of species. These are: the pollen should be able to successfully germinate on the stigma and the pollen tube should be able to grow down the style. Failure in any one of these can result in failure of intra- or interspecific hybridization (Reiseberg and Carney, 1998). The relative sizes of the pollen grain and the papillae of the stigma influence the successful germination of pollen grains. The presence of an interference zone in the style (which prevents the further growth of the "foreign" pollen) and the length of the style affect successful growth of the pollen tube down the style. In addition to these mechanical barriers, physiological and genetic incompatibility mechanisms play roles in successful hybridization. The post-zygotic barriers do also act in preventing successful interspecific hybridization. In this study pollen tube growth assessment was performed for both intra-and interspecific crosses. The results showed that cross-pollinating different plants of the same species always resulted in successful pollen tube growth. Intraspecific crosses were the next groups on the basis of pollen tube growth. Only few interspecific crosses resulted in successful pollen tube growth. This is in agreement with Evans (1962) who conducted both interspecific and intraspecific crosses in various species of *Trifolium* and found relatively higher rate of pollen tube growth in the intraspecific crosses.

Evans (1962) reported that there was generally no failure of the pollen tube growth in all the crosses even though good pollen tube growth was observed only in four of the interspecific crosses: *T. repens* x *T. uniflorum*; *T. repens* x *T. nigrescens*; *T. hybridum* x *T. ambiguum* and *T. pratens* x *T. medium*. Pandey (1955; cited in Evans, 1962), on the other hand, showed that inhibition of the pollen tube in the style is one important barrier in preventing species hybridization in the genus *Trifolium*. Absence of pollen- tube growth in some of the crosses of this study showed that some incompatibility mechanisms may work in preventing successful hybridization. In addition to the intrinsic incompatibility mechanisms, external factors like $p^H$ and temperature could affect pollen tube growth. Tuinstra and Wedel (2000), for example, observed the effect of incubation temperature on pollen germination of Sorghum and found out that pollen germination was not affected by changes in temperature between 20 and 40°C, but germination was significantly reduced at 10°C.

A high degree of variation was observed in flowering time among the accessions. The variation was observed both between species and among accessions of the same species. This was also reported by Kahurananga and Asres Tsehay (1991). Hence, optimization of the exact flowering times of the accessions (accessions to be hybridized) is very important before conducting hybridization experiments.

Before conducting the interspecific hybridization, the effect of bagging on seed formation was analyzed. The number of seeds produced by plants whose flowers were bagged (when hybridized) was compared with control plants that were left to set seed under normal conditions. Statistical analyses showed that there was no statistically significant difference among the controls and the bagged counterparts. This showed that the inter- and intraspecific hybridizations were not significantly affected by bagging.

Synchronization of the time of pollen shedding and stigma receptivity is another important parameter in relation to hybridization. Results of this study showed that there was a good level of intra- and inter-species compatibility (as was shown by the positive correlation) in the sizes of the flowers at which the pollen was shed and the stigma was receptive. This is true usually for self- pollinating species and shows that both *T. quartinianum* and *T. steudneri* are largely self-pollinating (Kahurananga, 1987). This is also in agreement with earlier works done in ILRI (formerly ILCA) (ILCA, 1991).

The results of studies on pollen viability showed that pollen viability decreases with time. The pollen starts to shed usually in the morning. The number of pollen grains that fluoresce to the FDA decrease with time. Fluorescence is caused when the FDA solution enters the cell's cytoplasm resulting in the release of fluorescin as a result of enzymatic cleavage (Shivnanna and Johri, 1985). The plasma lemma of dead cells can not hold the FDA solution. Moreover, the enzymes in these cells have reduced activity. Hence, dead cells are not endowed with the fluorescence observed in live cells. The presence of intact plasma lemma is a prerequisite for fluorescence (Heslop-Harisson and Heslop-Harisson, 1970). Immature and old buds do not have intact plasma lemma resulting in no or reduced fluorescence. The decrease in the number of fluorescing pollen grains shows that the number of viable pollen grains decreases with time, possibly because some of the pollen grains die. Hence it was necessary to look for the time of the day when the maximum amounts of live pollen grains could be obtained before conducting hybridization. The results of this study showed that early mornings (9.00- 11.00 am) were the ideal times to obtain the maximum amount of live pollen grains.

Some of the crossing experiments (that involved both similar and different species combinations) resulted seed formation. This showed that there is some level of compatibility

among the involved species. The seeds formed, however, were shriveled and of relatively smaller size. This was also observed by Guravich and Trimble (1949; cited in Evans, 1962) and Pritchard and Mannetje (1967) who performed some interspecific crosses and found seeds of these natures. In most of the crosses, however, no seeds were formed. This is in agreement with the results of Wexelsen (1928; cited in Evans, 1962) and Evans (1962) that showed the presence of high degree of incompatibility among species of *Trifolium.* A low rate of out-crossing is also reported (Kittelson and Maron, 2000). However, some success in interspecific hybridization in some species of *Trifolium* is reported by some other workers. Pritchard and Mannetje (1967), for example, reported that *T. semiplosum, T. masaiensee, T. rueppelianum* var. *rueppelianum* and *T. pseudostriatum* are cross-compatible. Seeds were produced by interspecific hybridization of these species, even though the seeds produced were shriveled. In another setting, fourteen species pairs and their reciprocals were inter-pollinated; the species being paired according to their taxonomic affinities. In all, twelve interspecific crosses were made within the tribes. Of the 28 hybridizations made, seeds were obtained in 3 species combinations and 2 of their reciprocals; they were shriveled and showed very poor germination capacity. No mature hybrids were obtained (Evans, 1962). In the present study, six of the interspecific hybridization trials resulted in seed formation while two did not produce seeds.

## 5.3. Cytogenetics

There was no difference in the chromosome number of the three taxa. Chromosome number, hence, could not be used to identify and characterize the distinct morphotype. This agrees to the work of Wexelsem (1928; cited in El-kholy, 1990) who stated that chromosome variation (either in terms of size or number) can not be used for classification of the genus. The chromosomes of these species were very small and were not easily observable. Hence, more sensitive techniques need to be used to get the whole picture of the chromosome profiles of both these species and the whole genus.

# 6. CONCLUSIONS AND RECOMMENDATIONS

In general, the two species (*T. quartinianum* and *T. steudneri)* showed overlaps on some of the morphological characteristics. This may be because these characters are characters defining the whole genus than individual species. On certain characteristics, these species showed significant levels of differences. These and other key characters can be looked for to serve as identifying markers for these species.

The distinct morphotype, on the other hand, exhibited certain levels of similarities and differences to the species which, on average, makes it different from both. This is revealed in the dendrogram constructed based on these characters. This variation and distinct nature can be indicative of the fact that the distinct morphotype can be a member of a different taxon; misclassified with *T. steudneri.*

Most of the agriculturally important species of *Trifolium* are highly self- incompatible and extremely heterozygous. Agriculturally important characters, like winter hardiness, the perennial habit and resistance to some of the most serious clover diseases are found in some species, but not in others. Hence there is a great need to incorporate these agriculturally important features into the agriculturally important varieties (Evans, 1962). Interspecific hybridization helps a great deal in introducing such characters into the agriculturally important varieties and obtaining improved varieties of desired features. The success in interspecific hybridization is indicative of the potentials in this line. The hybrids can be studied for the pattern of growth and chromosome behavior (e.g. chromosome pairing) during times of meiosis.

The distinct morphotype produced seeds with some accessions of both species. The seeds were, however, smaller in size and shriveled. Large-scale breeding experiments need to be done to come up with a generalized picture of the breeding system of the parent species and the hybrids formed. The positive inter-species correlations in the sizes of the flowers for pollen shedding and stigma receptivity also indicate that these taxa may hybridize under natural conditions. The geographic distribution of the three taxa can be assessed to see if there exists an overlap in their geographic ranges and if there are natural hybrids of these species (Ellstrad *et al.*, 1996). The hybrid seeds and the plants obtained thereafter can be tested for their germination ability, morphology and chromosome pairing.

Ethiopia is believed to be one of the main centers of origin of the genus *Trifolium*. The overall economy of the country is dependent on agriculture. The country is also known to have a great deal of potential in line with livestock production. *Trifolium*, as one of the forage genus, helps a great deal in maintaining and improving agricultural productivity. For example, intercropping of many species of *Trifolium* with many cereals resulted in increased productivity (Haque *et al.*, 2000; Mpairwe *et al.*, 2002). In order to utilize the potentials in these forage species one needs to have enough knowledge about the level of diversity in the forage species. It seems, hence, very demanding to have studies with this regard.

Further molecular characterizations (e.g. RAPD, RFLP or mini- or microsatelite typing) and detailed cytogenetic analyses, can better reveal the real picture of the kind and level of variation that exists among the taxa (Williams *et al.*, 2001). This kind of analyses can also be done on the accessions that were found deviating from the rest of the accessions.

In conclusion, a species, according to Mayr (1970), is a reproductively isolated aggregate of interbreeding population. This concept indicates that a species consists of groups of actually or potentially interbreeding populations genetically isolated from other groups by one or more reproductive isolating mechanisms. The two species should, basically, be distinct to each other on most of the characters. The fact that these species are closer on the morphological characters may be indicative of the fact that they may be members of a larger species complex with relatively wider ranges of adaptable characters and locally interbreeding populations. The hybridization experiments showed that certain accessions of these species interbreed on at least the artificial hybridization trials. The observed hybridization potential of these species points out that the interspecific reproductive barriers are either surpassed or do not exist. Therefore one needs to question whether these taxa belong to different groups or are members of a larger taxonomic unit.

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# 8. APPENDIX

Appendix 1. Average (Mean ± SD) values of all Accessions.

| Accessions | | PHT | HSW | WSL | LSL | LWRS | LTL | WTL | LWRT | NPI | NSP | NSPB | LCL | LOC | SPS | SSR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10059 | mean | 30.20 | 0.12 | 1.00 | 5.20 | 5.31 | 4.86 | 0.74 | 6.59 | 8.20 | 1.80 | 1.80 | 0.88 | 1.58 | 8.00 | 8.80 |
| | SD | 4.49 | 0.01 | 0.16 | 0.43 | 1.02 | 0.09 | 0.05 | 0.44 | 0.45 | 1.10 | 0.45 | 0.04 | 0.08 | 1.87 | 1.48 |
| 11056 | mean | 31.80 | 0.17 | 0.86 | 3.02 | 3.52 | 5.44 | 1.38 | 3.97 | 5.60 | 3.40 | 2.20 | 0.82 | 1.50 | 5.20 | 6.40 |
| | SD | 1.30 | 0.01 | 0.13 | 0.45 | 0.17 | 0.48 | 0.11 | 0.54 | 1.95 | 0.89 | 0.84 | 0.08 | 0.10 | 1.68 | 1.14 |
| 13716 | mean | 23.00 | 0.19 | 1.02 | 4.94 | 4.89 | 5.46 | 1.16 | 4.72 | 8.80 | 1.80 | 4.40 | 0.88 | 1.48 | 4.00 | 3.92 |
| | SD | 2.55 | 0.01 | 0.13 | 0.25 | 0.49 | 0.51 | 0.11 | 0.30 | 1.79 | 0.45 | 1.82 | 0.04 | 0.04 | 0.71 | 0.48 |
| 13808 | mean | 26.60 | 0.16 | 1.32 | 4.36 | 3.35 | 4.48 | 1.36 | 3.38 | 25.80 | 1.80 | 1.00 | 0.90 | 1.44 | 4.70 | 4.42 |
| | SD | 1.14 | 0.01 | 0.27 | 0.55 | 0.25 | 0.64 | 0.30 | 0.65 | 3.27 | 0.45 | 0.00 | 0.01 | 0.05 | 0.84 | 1.28 |
| 6297 | mean | 43.20 | 0.14 | 1.12 | 4.42 | 3.96 | 4.82 | 1.36 | 3.57 | 15.76 | 2.32 | 2.90 | 0.88 | 1.50 | 3.90 | 4.34 |
| | SD | 1.92 | 0.01 | 0.11 | 0.56 | 0.46 | 0.48 | 0.09 | 0.58 | 0.22 | 0.30 | 0.07 | 0.04 | 0.07 | 0.89 | 0.65 |
| 6301 | mean | 41.40 | 0.12 | 0.86 | 3.96 | 4.61 | 3.96 | 0.88 | 4.51 | 12.70 | 1.40 | 0.00 | 0.79 | 1.72 | 4.20 | 8.00 |
| | SD | 15.39 | 0.01 | 0.05 | 0.26 | 0.13 | 0.11 | 0.04 | 0.24 | 5.43 | 0.55 | 0.00 | 0.05 | 0.09 | 2.17 | 0.00 |
| 7693 | mean | 37.40 | 0.13 | 1.28 | 4.36 | 3.51 | 4.68 | 1.48 | 3.22 | 5.60 | 4.20 | 0.00 | 0.82 | 1.64 | 4.40 | 4.80 |
| | SD | 1.52 | 0.02 | 0.24 | 0.23 | 0.69 | 0.36 | 0.26 | 0.51 | 0.89 | 0.45 | 0.00 | 0.04 | 0.11 | 0.65 | 1.10 |
| 8452 | mean | 24.20 | 0.14 | 0.74 | 4.50 | 6.13 | 4.18 | 0.78 | 5.34 | 10.00 | 3.00 | 0.00 | 0.92 | 1.64 | 3.90 | 4.30 |
| | SD | 4.09 | 0.01 | 0.05 | 0.30 | 0.82 | 1.08 | 0.04 | 1.23 | 3.08 | 0.71 | 0.00 | 0.02 | 0.13 | 0.89 | 0.67 |
| 8535 | mean | 37.80 | 0.14 | 1.10 | 3.60 | 3.27 | 3.94 | 1.44 | 2.74 | 22.80 | 4.00 | 0.00 | 0.82 | 1.52 | 4.62 | 4.52 |
| | SD | 9.91 | 0.01 | 0.00 | 0.14 | 0.13 | 0.15 | 0.09 | 0.24 | 0.45 | 0.00 | 0.00 | 0.04 | 0.08 | 0.91 | 0.57 |
| 9455 | mean | 44.80 | 0.16 | 1.16 | 4.98 | 4.42 | 4.64 | 1.18 | 5.72 | 8.40 | 4.40 | 5.60 | 0.82 | 1.54 | 4.78 | 4.18 |
| | SD | 9.83 | 0.01 | 0.28 | 0.46 | 0.66 | 0.75 | 0.50 | 5.40 | 0.89 | 0.55 | 0.55 | 0.02 | 0.05 | 1.70 | 0.56 |
| 10125 | mean | 40.80 | 0.15 | 0.98 | 4.58 | 4.70 | 5.22 | 1.10 | 4.75 | 8.20 | 2.20 | 2.80 | 0.52 | 0.92 | 4.80 | 6.10 |
| | SD | 3.83 | 0.01 | 0.08 | 0.24 | 0.41 | 0.27 | 0.07 | 0.26 | 2.05 | 0.84 | 0.84 | 0.04 | 0.04 | 1.30 | 0.22 |
| 7658 | mean | 41.80 | 0.16 | 0.94 | 5.32 | 5.67 | 5.40 | 1.00 | 5.43 | 13.00 | 1.40 | 3.00 | 0.44 | 0.76 | 5.80 | 6.50 |
| | SD | 1.64 | 0.01 | 0.05 | 0.60 | 0.70 | 0.55 | 0.10 | 0.64 | 2.24 | 0.55 | 0.71 | 0.11 | 0.05 | 1.60 | 0.87 |
| 7659 | mean | 33.40 | 0.15 | 1.04 | 4.90 | 4.81 | 4.56 | 1.06 | 4.32 | 7.08 | 2.08 | 2.32 | 0.46 | 0.76 | 5.40 | 6.80 |
| | SD | 3.13 | 0.01 | 0.13 | 0.34 | 0.96 | 0.46 | 0.05 | 0.56 | 0.23 | 0.08 | 0.08 | 0.05 | 0.05 | 0.96 | 0.84 |
| 8461 | mean | 29.20 | 0.14 | 0.94 | 4.32 | 4.61 | 4.56 | 1.12 | 4.08 | 13.80 | 1.60 | 2.80 | 0.50 | 0.90 | 5.20 | 6.66 |
| | SD | 1.64 | 0.01 | 0.05 | 0.22 | 0.32 | 0.27 | 0.08 | 0.18 | 1.10 | 0.55 | 0.84 | 0.07 | 0.07 | 1.04 | 0.42 |
| 8485 | mean | 33.60 | 0.15 | 0.82 | 3.76 | 4.59 | 3.70 | 0.84 | 4.41 | 8.40 | 3.40 | 2.40 | 0.40 | 0.80 | 4.58 | 7.26 |
| | SD | 5.37 | 0.01 | 0.08 | 0.65 | 0.64 | 0.44 | 0.05 | 0.48 | 1.82 | 0.89 | 1.67 | 0.16 | 0.07 | 1.49 | 0.70 |
| 9058 | mean | 36.60 | 0.15 | 0.96 | 4.62 | 4.81 | 4.46 | 0.98 | 4.74 | 12.20 | 3.00 | 2.20 | 0.48 | 0.80 | 6.90 | 7.40 |
| | SD | 1.82 | 0.02 | 0.05 | 0.40 | 0.28 | 0.40 | 0.24 | 1.01 | 5.22 | 0.00 | 0.45 | 0.08 | 0.00 | 1.43 | 0.89 |
| 9452 | mean | 44.60 | 0.10 | 1.20 | 3.74 | 3.12 | 4.18 | 1.40 | 2.98 | 12.40 | 2.60 | 2.00 | 0.50 | 0.88 | 3.84 | 4.40 |
| | SD | 0.89 | 0.00 | 0.10 | 0.31 | 0.07 | 0.43 | 0.07 | 0.18 | 0.55 | 0.89 | 0.00 | 0.00 | 0.04 | 0.15 | 0.89 |
| 9720 | mean | 35.20 | 0.14 | 0.88 | 4.80 | 5.46 | 3.84 | 0.88 | 4.38 | 15.80 | 2.40 | 2.20 | 0.12 | 0.90 | 6.90 | 6.40 |
| | SD | 1.64 | 0.01 | 0.04 | 0.07 | 0.31 | 0.42 | 0.04 | 0.54 | 2.17 | 0.55 | 1.30 | 0.04 | 0.07 | 1.43 | 1.14 |
| 9956 | mean | 32.40 | 0.14 | 0.74 | 4.36 | 6.06 | 4.88 | 0.78 | 6.29 | 8.80 | 2.00 | 2.40 | 0.44 | 0.80 | 4.50 | 7.80 |
| | SD | 2.51 | 0.01 | 0.11 | 0.47 | 1.40 | 0.27 | 0.04 | 0.64 | 2.68 | 0.00 | 0.89 | 0.05 | 0.07 | 1.17 | 1.35 |
| 9966 | mean | 32.60 | 0.13 | 0.96 | 4.56 | 4.77 | 4.28 | 1.04 | 4.14 | 6.60 | 3.00 | 2.00 | 0.48 | 0.88 | 6.80 | 7.20 |
| | SD | 3.44 | 0.01 | 0.05 | 0.27 | 0.45 | 0.60 | 0.11 | 0.63 | 1.95 | 1.22 | 0.00 | 0.04 | 0.04 | 1.64 | 0.45 |
| 9991 | mean | 40.00 | 0.12 | 1.00 | 4.54 | 4.54 | 4.14 | 0.98 | 4.22 | 9.20 | 3.00 | 2.00 | 0.50 | 0.80 | 4.98 | 5.80 |
| | SD | 1.87 | 0.01 | 0.10 | 0.56 | 0.20 | 0.50 | 0.08 | 0.38 | 3.42 | 0.71 | 0.00 | 0.07 | 0.07 | 1.20 | 0.45 |

Appendix 2. One way ANOVA for all characters used for all accessions.

Tests of Between-Subjects Effects

| Source | Dependent Variable | Type I SS | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| SPECIES | PHT | 515.09 | 2 | 257.55 | 9.34 | 0.00 |
| | HSW | 0.01 | 2 | 0.00 | 40.01 | 0.00 |
| | WSLTOT | 0.58 | 2 | 0.29 | 18.82 | 0.00 |
| | LSLTOT | 3.90 | 2 | 1.95 | 12.33 | 0.00 |
| | RATTOT | 23.61 | 2 | 11.80 | 32.42 | 0.00 |
| | LTLTOT | 1.25 | 2 | 0.62 | 2.72 | 0.07 |
| | WTLTOT | 1.48 | 2 | 0.74 | 32.24 | 0.00 |
| | RATTOT_1 | 13.57 | 2 | 6.78 | 4.11 | 0.02 |
| | NPITOT | 111.26 | 2 | 55.63 | 9.00 | 0.00 |
| | NSPTOT | 4.08 | 2 | 2.04 | 4.57 | 0.01 |
| | NSPBTOT | 9.72 | 2 | 4.86 | 8.61 | 0.00 |
| | LCLTOT | 4.47 | 2 | 2.24 | 547.36 | 0.00 |
| | LOCTOT | 13.56 | 2 | 6.78 | 1255.34 | 0.00 |
| | SPS | 25.17 | 2 | 12.59 | 10.36 | 0.00 |
| | SSR | 64.13 | 2 | 32.07 | 46.31 | 0.00 |
| ACC_NUM | PHT | 3640.64 | 20 | 202.26 | 7.33 | 0.00 |
| | HSW | 0.03 | 20 | 0.00 | 12.57 | 0.00 |
| | WSLTOT | 1.94 | 20 | 0.11 | 7.03 | 0.00 |
| | LSLTOT | 27.33 | 20 | 1.52 | 9.61 | 0.00 |
| | RATTOT | 53.39 | 20 | 2.97 | 8.15 | 0.00 |
| | LTLTOT | 26.60 | 20 | 1.48 | 6.44 | 0.00 |
| | WTLTOT | 4.10 | 20 | 0.23 | 9.95 | 0.00 |
| | RATTOT_1 | 89.46 | 20 | 4.97 | 3.01 | 0.00 |
| | NPITOT | 2664.33 | 20 | 148.02 | 23.96 | 0.00 |
| | NSPTOT | 77.85 | 20 | 4.33 | 9.68 | 0.00 |
| | NSPBTOT | 186.27 | 20 | 10.35 | 18.34 | 0.00 |
| | LCLTOT | 0.68 | 20 | 0.04 | 9.29 | 0.00 |
| | LOCTOT | 0.51 | 20 | 0.03 | 5.20 | 0.00 |
| | SPS | 107.73 | 20 | 5.99 | 4.93 | 0.00 |
| | SSR | 154.54 | 20 | 8.59 | 12.40 | 0.00 |
| REPLICA | PHT | 77.87 | 4 | 19.47 | 0.71 | 0.59 |
| | HSW | 0.00 | 4 | 0.00 | 1.40 | 0.24 |
| | WSLTOT | 0.23 | 4 | 0.06 | 3.69 | 0.01 |
| | LSLTOT | 0.92 | 4 | 0.23 | 1.46 | 0.22 |
| | RATTOT | 1.62 | 4 | 0.40 | 1.11 | 0.36 |
| | LTLTOT | 2.03 | 4 | 0.51 | 2.21 | 0.08 |
| | WTLTOT | 0.42 | 4 | 0.11 | 4.62 | 0.00 |
| | RATTOT_1 | 10.62 | 4 | 2.65 | 1.61 | 0.18 |
| | NPITOT | 15.13 | 4 | 3.78 | 0.61 | 0.66 |
| | NSPTOT | 0.25 | 4 | 0.06 | 0.14 | 0.97 |
| | NSPBTOT | 2.51 | 4 | 0.63 | 1.11 | 0.36 |
| | LCLTOT | 0.02 | 4 | 0.01 | 1.39 | 0.24 |
| | LOCTOT | 0.02 | 4 | 0.00 | 0.86 | 0.49 |
| | SPS | 47.35 | 4 | 11.84 | 9.75 | 0.00 |
| | SSR | 6.07 | 4 | 1.52 | 2.19 | 0.08 |
| Error | PHT | 2206.93 | 80 | 27.59 | | |
| | HSW | 0.01 | 80 | 0.00 | | |
| | WSLTOT | 1.23 | 80 | 0.02 | | |
| | LSLTOT | 12.64 | 80 | 0.16 | | |
| | RATTOT | 29.13 | 80 | 0.36 | | |
| | LTLTOT | 18.35 | 80 | 0.23 | | |
| | WTLTOT | 1.83 | 80 | 0.02 | | |
| | RATTOT_1 | 132.01 | 80 | 1.65 | | |
| | NPITOT | 494.27 | 80 | 6.18 | | |
| | NSPTOT | 35.74 | 80 | 0.45 | | |
| | NSPBTOT | 45.14 | 80 | 0.56 | | |
| | LCLTOT | 0.33 | 80 | 0.00 | | |
| | LOCTOT | 0.43 | 80 | 0.01 | | |
| | SPS | 97.17 | 80 | 1.21 | | |
| | SSR | 55.39 | 80 | 0.69 | | |
| Total | PHT | 6440.53 | 104 | | | |
| | HSW | 0.05 | 104 | | | |
| | WSLTOT | 3.98 | 104 | | | |
| | LSLTOT | 44.79 | 104 | | | |
| | RATTOT | 107.74 | 104 | | | |
| | LTLTOT | 48.22 | 104 | | | |
| | WTLTOT | 7.83 | 104 | | | |
| | RATTOT_1 | 245.65 | 104 | | | |
| | NPITOT | 3284.99 | 104 | | | |
| | NSPTOT | 117.93 | 104 | | | |

Appendix 3. Homogenous subsets generated for the accessions at each trait.

**HSW**

| | SPECIES | N | Subset for alpha = .05 | |
|---|---|---|---|---|
| | | | 1 | 2 |
| Tukey HSD | DM (9452) | 5 | .1000 | |
| | T. steudneri | 48 | | .1425 |
| | T. quartinianum | 50 | | .1466 |
| | Sig. | | 1.000 | .861 |

Means for groups in homogeneous subsets are displayed.

**WSLTOT**

| | SPECIES | N | Subset for alpha = .05 | |
|---|---|---|---|---|
| | | | 1 | 2 |
| Tukey HSD | T. steudneri | 50 | .9260 | |
| | T. quartinianum | 50 | 1.0460 | 1.0460 |
| | DM (9452) | 5 | | 1.2000 |
| | Sig. | | .232 | .093 |

Means for groups in homogeneous subsets are displayed.

**LSLTOT**

| | SPECIES | N | Subset for alpha = .05 | |
|---|---|---|---|---|
| | | | 1 | 2 |
| Tukey HSD | DM (9452) | 5 | 3.7400 | |
| | T. quartinianum | 50 | 4.3340 | 4.3340 |
| | T. steudneri | 50 | | 4.5760 |
| | Sig. | | .054 | .607 |

Means for groups in homogeneous subsets are displayed.

**PHT**

| | SPECIES | N | Subset for alpha = .05 | |
|---|---|---|---|---|
| | | | 1 | 2 |
| Tukey HSD | T. quartinianum | 50 | 34.0400 | |
| | T. steudneri | 50 | 35.5600 | |
| | DM (9452) | 5 | | 44.6000 |
| | Sig. | | .872 | 1.000 |

Means for groups in homogeneous subsets are displayed.

RATTOT

| | SPECIES | N | Subset for alpha = .05 | |
|---|---|---|---|---|
| | | | 1 | 2 |
| Tukey HSD | DM (9452) | 5 | 3.1180 | |
| | T. quartinianum | 50 | | 4.2954 |
| | T. steudneri | 50 | | 5.0012 |
| | Sig. | | 1.000 | .132 |

Means for groups in homogeneous subsets are displayed.

LTLTOT

| | SPECIES | N | Subset for alpha = .05 |
|---|---|---|---|
| | | | 1 |
| Tukey HSD | DM (9452) | 5 | 4.1800 |
| | T. steudneri | 50 | 4.5040 |
| | T. quartinianum | 50 | 4.6460 |
| | Sig. | | .204 |

Means for groups in homogeneous subsets are displayed.

WTLTOT

| | SPECIES | N | Subset for alpha = .05 | |
|---|---|---|---|---|
| | | | 1 | 2 |
| Tukey HSD | T. steudneri | 50 | .9780 | |
| | T. quartinianum | 50 | 1.1760 | 1.1760 |
| | DM (9452) | 5 | | 1.4000 |
| | Sig. | | .122 | .069 |

Means for groups in homogeneous subsets are displayed.

RATTOT_1

| | SPECIES | N | Subset for alpha = .05 | |
|---|---|---|---|---|
| | | | 1 | 2 |
| Tukey HSD | DM (9452) | 5 | 2.9820 | |
| | T. quartinianum | 50 | 4.3770 | 4.3770 |
| | T. steudneri | 50 | | 4.6748 |
| | Sig. | | .059 | .875 |

Means for groups in homogeneous subsets are displayed.

NPITOT

| | SPECIES | N | Subset for alpha = .05 |
|---|---|---|---|
| | | | 1 |
| Tukey HSD | T. steudneri | 50 | 10.3080 |
| | DM (9452) | 5 | 12.4000 |
| | T. quartinianum | 46 | 12.6152 |
| | Sig. | | .546 |

Means for groups in homogeneous subsets are displayed.

NSPTOT

| | SPECIES | N | Subset for alpha = .05 |
|---|---|---|---|
| | | | 1 |
| Tukey HSD | T. steudneri | 50 | 2.4080 |
| | DM (9452) | 5 | 2.6000 |
| | T. quartinianum | 46 | 2.6870 |
| | Sig. | | .779 |

Means for groups in homogeneous subsets are displayed.

NSPBTOT

| | SPECIES | N | Subset for alpha = .05 |
|---|---|---|---|
| | | | 1 |
| Tukey HSD | T. quartinianum | 46 | 1.9457 |
| | DM (9452) | 5 | 2.0000 |
| | T. steudneri | 50 | 2.4120 |
| | Sig. | | .719 |

Means for groups in homogeneous subsets are displayed.

LCLTOT

| | SPECIES | N | Subset for alpha = .05 | |
|---|---|---|---|---|
| | | | 1 | 2 |
| Tukey HSD | T. steudneri | 50 | .4342 | |
| | DM (9452) | 5 | .5000 | |
| | T. quartinianum | 49 | | .8555 |
| | Sig. | | .232 | 1.000 |

Means for groups in homogeneous subsets are displayed.

LOCTOT

| | SPECIES | N | Subset for alpha = .05 | |
|---|---|---|---|---|
| | | | 1 | 2 |
| Tukey HSD | T. steudneri | 50 | .8320 | |
| | DM (9452) | 5 | .8800 | |
| | T. quartinianum | 49 | | 1.5506 |
| | Sig. | | .413 | 1.000 |

Means for groups in homogeneous subsets are displayed.

SPS

| | SPECIES | N | Subset for alpha = .05 |
|---|---|---|---|
| | | | 1 |
| Tukey HSD | DM (9452) | 5 | 3.8400 |
| | T. quartinianum | 46 | 4.6413 |
| | T. steudneri | 37 | 5.2378 |
| | Sig. | | .071 |

Means for groups in homogeneous subsets are displayed.

SSR

| | SPECIES | N | Subset for alpha = .05 | |
|---|---|---|---|---|
| | | | 1 | 2 |
| Tukey HSD | DM (9452) | 5 | 4.4000 | |
| | T. quartinianum | 46 | 5.4217 | 5.4217 |
| | T. steudneri | 37 | | 6.8946 |
| | Sig. | | .238 | .054 |

Means for groups in homogeneous subsets are displayed.

Appendix 6. Wilk's Lambda and Eigen values for all the accessions.

**Wilks' Lambda**

| Step | Number of Variables | Lambda | df1 | df2 | df3 | Exact F | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Statistic | df1 | df2 | Sig. |
| 1 | 1 | .814 | 1 | 2 | 81 | 9.262 | 2 | 81.000 | 2.386E-04 |
| 2 | 2 | .051 | 2 | 2 | 81 | 137.695 | 4 | 160.000 | .000 |
| 3 | 3 | .029 | 3 | 2 | 81 | 127.547 | 6 | 158.000 | .000 |
| 4 | 4 | .023 | 4 | 2 | 81 | 109.065 | 8 | 156.000 | .000 |

**Eigenvalues**

| Function | Eigenvalue | % of Variance | Cumulative % | Canonical Correlation |
|---|---|---|---|---|
| 1 | 25.762[a] | 97.6 | 97.6 | .981 |
| 2 | .624[a] | 2.4 | 100.0 | .620 |

a. First 2 canonical discriminant functions were used in the analysis.

Appendix 7. Paired Euclidean Distance among all the Accessions Calculated Based on the Morphological Characters.

**Proximity Matrix**

| Case | Euclidean Distance | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TQ629 | TQ6301 | TQ7693 | TQ8452 | TQ8535 | TQ9455 | TQ10059 | TQ11056 | TQ13808 | TQ13716 | TS7658 | TS7659 | TS8485 | TS9058 | TS9720 | TS9956 | TS9966 | TS9991 | TS10125 | TS8461 | TS94 |
| TQ6297 | | 1.29 | 2.14 | 4.69 | 4.72 | 4.82 | 6.86 | 1.56 | 2.95 | 3.52 | 1.87 | .654 | 2.18 | 1.47 | 1.55 | 4.44 | 3.38 | 1.83 | 2.33 | 7.46 | 8 |
| TQ6301 | 1.29 | | 1.16 | 4.03 | 3.93 | 4.19 | 5.61 | 1.65 | 1.96 | 2.39 | 1.43 | 1.28 | 1.35 | .737 | 1.17 | 3.38 | 3.24 | 1.61 | 1.53 | 6.25 | 7 |
| TQ7693 | 2.14 | 1.16 | | 3.69 | 3.29 | 3.91 | 4.86 | 2.71 | 2.32 | 2.06 | 1.27 | 2.24 | 1.24 | 1.27 | 1.46 | 3.67 | 4.09 | 1.92 | 2.03 | 5.71 | 6 |
| TQ8452 | 4.69 | 4.03 | 3.69 | | 1.66 | .912 | 5.03 | 5.06 | 3.52 | 2.97 | 4.78 | 4.59 | 4.74 | 3.70 | 4.93 | 4.96 | 5.06 | 4.03 | 4.43 | 4.52 | 8 |
| TQ8535 | 4.72 | 3.93 | 3.29 | 1.66 | | 1.54 | 4.35 | 5.31 | 3.39 | 2.40 | 4.44 | 4.80 | 4.45 | 3.80 | 4.71 | 5.08 | 5.87 | 4.37 | 4.61 | 4.46 | 7 |
| TQ9455 | 4.82 | 4.19 | 3.91 | .912 | 1.54 | | 5.18 | 5.20 | 3.37 | 2.85 | 5.05 | 4.79 | 4.99 | 3.94 | 5.16 | 5.08 | 5.34 | 4.41 | 4.69 | 4.71 | 8 |
| TQ10059 | 6.86 | 5.61 | 4.86 | 5.03 | 4.35 | 5.18 | | 6.73 | 4.84 | 3.84 | 5.72 | 6.79 | 5.20 | 5.61 | 5.92 | 4.42 | 7.03 | 5.98 | 5.36 | 2.25 | 3 |
| TQ11056 | 1.56 | 1.65 | 2.71 | 5.06 | 5.31 | 5.20 | 6.73 | | 2.68 | 3.60 | 2.56 | 1.14 | 2.31 | 1.71 | 1.96 | 3.43 | 2.17 | 1.90 | 1.54 | 7.15 | 8 |
| TQ13808 | 2.95 | 1.96 | 2.32 | 3.52 | 3.39 | 3.37 | 4.84 | 2.68 | | 1.30 | 3.17 | 2.85 | 2.75 | 2.14 | 2.97 | 2.51 | 3.48 | 2.98 | 2.27 | 5.17 | 7 |
| TQ13716 | 3.52 | 2.39 | 2.06 | 2.97 | 2.40 | 2.85 | 3.84 | 3.60 | 1.30 | | 3.19 | 3.52 | 2.82 | 2.49 | 3.22 | 3.00 | 4.42 | 3.27 | 2.80 | 4.38 | 6 |
| TS7658 | 1.87 | 1.43 | 1.27 | 4.78 | 4.44 | 5.05 | 5.72 | 2.56 | 3.17 | 3.19 | | 2.03 | .915 | 1.71 | .711 | 4.15 | 4.22 | 2.09 | 2.25 | 6.72 | 7 |
| TS7659 | .654 | 1.28 | 2.24 | 4.59 | 4.80 | 4.79 | 6.79 | 1.14 | 2.85 | 3.52 | 2.03 | | 2.17 | 1.25 | 1.62 | 4.10 | 2.79 | 1.53 | 1.96 | 7.24 | 8 |
| TS8485 | 2.18 | 1.35 | 1.24 | 4.74 | 4.45 | 4.99 | 5.20 | 2.31 | 2.75 | 2.82 | .915 | 2.17 | | 1.60 | .807 | 3.37 | 3.84 | 1.96 | 1.59 | 6.19 | 6 |
| TS9058 | 1.47 | .737 | 1.27 | 3.70 | 3.80 | 3.94 | 5.61 | 1.71 | 2.14 | 2.49 | 1.71 | 1.25 | 1.60 | | 1.46 | 3.43 | 2.97 | 1.07 | 1.36 | 6.08 | 7 |
| TS9720 | 1.55 | 1.17 | 1.46 | 4.93 | 4.71 | 5.16 | 5.92 | 1.96 | 2.97 | 3.22 | .711 | 1.62 | .807 | 1.46 | | 3.86 | 3.75 | 1.80 | 1.79 | 6.83 | 7 |
| TS9956 | 4.44 | 3.38 | 3.67 | 4.96 | 5.08 | 5.08 | 4.42 | 3.43 | 2.51 | 3.00 | 4.15 | 4.10 | 3.37 | 3.43 | 3.86 | | 3.31 | 3.83 | 2.49 | 4.62 | 6 |
| TS9966 | 3.38 | 3.24 | 4.09 | 5.06 | 5.87 | 5.34 | 7.03 | 2.17 | 3.48 | 4.42 | 4.22 | 2.79 | 3.84 | 2.97 | 3.75 | 3.31 | | 2.80 | 2.45 | 6.84 | 9 |
| TS9991 | 1.83 | 1.61 | 1.92 | 4.03 | 4.37 | 4.41 | 5.98 | 1.90 | 2.98 | 3.27 | 2.09 | 1.53 | 1.96 | 1.07 | 1.80 | 3.83 | 2.80 | . | 1.48 | 6.39 | 8 |
| TS10125 | 2.33 | 1.53 | 2.03 | 4.43 | 4.61 | 4.69 | 5.36 | 1.54 | 2.27 | 2.80 | 2.25 | 1.96 | 1.59 | 1.36 | 1.79 | 2.49 | 2.45 | 1.48 | | 5.85 | 7 |
| TS8461 | 7.46 | 6.25 | 5.71 | 4.52 | 4.46 | 4.71 | 2.25 | 7.15 | 5.17 | 4.38 | 6.72 | 7.24 | 6.19 | 6.08 | 6.83 | 4.62 | 6.84 | 6.39 | 5.85 | | 5 |
| TS9452 | 8.81 | 7.67 | 6.90 | 8.32 | 7.20 | 8.36 | 3.85 | 8.78 | 7.26 | 6.38 | 7.31 | 8.87 | 6.88 | 7.90 | 7.55 | 6.76 | 9.65 | 8.35 | 7.58 | 5.84 | |

This is a dissimilarity matrix