# CHARACTERIZATION AND EVALUATION OF *INDIGOFERA* SPECIES AS POTENTIAL FORAGE AND COVER CROPS FOR SEMI-ARID AND ARID ECOSYSTEMS

**by**

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***"I have dedicated this dissertation in memory of my beloved mother, Kedija Seid, and my father, the late Hassen Imamu, who did every thing they could to see us getting education at a higher level."***

## DECLARATION

I declare that this dissertation, submitted for the degree of Doctor of Philosophy in Pasture Science at the University of Pretoria, is my own work and has not been previously submitted by me for a degree at another University.

Signature _____

Abubeker Hassen

## PREFACE

This work was conducted in the Department of Plant Production and Soil Science, University of Pretoria, South Africa. The project involved field experiments, glasshouse studies and laboratory trials aimed at the characterization and evaluation of *Indigofera* accessions as potential forage and cover crops for semi-arid and arid ecological areas.

This dissertation is based on the following chapters, which have been published, accepted or submitted for publication. The dissertation is prepared in accordance to the guidelines set-up for authors for publication of manuscripts in the journal of Tropical Grassland.

1. **Abubeker Hassen, P.A. Pieterse and N.F.G. Rethman** (2004) Effect of pre-planting seed treatment on dormancy breaking and germination of *Indigofera* accessions (*published in Tropical Grasslands, Volume 38, No 3, page 154-157*).
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*Indigofera* species subjected to moisture stress and non-stress conditions (*to be submitted for publication in Journal of Arid Environments*).

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## LIST OF ABBREVIATIONS

3-NPA: 3-nitropropionic acid.

AOAC: Association of Official Analytical chemists.

ARC: Agricultural Research Council.

ARTP: Agricultural research training project.

asl: Above sea level.

$BW^{-0.75}$: Metabolic body weight.

CIAT: Centro Internacional de Agricultura Tropical.

CP: Crude protein.

CPD%: Crude protein digestibility.

CPI: Crude protein intake.

CSDav: Mean canopy spread diameter.

CSDm: Canopy spread diameter at maximum point.

CSDr: Canopy spread diameter at right angle to the maximum.

Cu: Copper.

DABA: 2,4-diaminobutric acid.

DATI: Drought stress tolerance indices.

DCPI: Digestible crude protein intake.

DFF: Days to first flowering.

DFPF: Days to 50% flowering.

DHP: 3-hydroxy-4(1H)-pyridone.

DM: Dry matter.

DMI: Dry matter intake.

DNDFI: Digestible neutral detergent fibre intake.

DOMI: Digestible organic matter intake.

$DSTI_{LDM}$: DSTI computed based on Leaf dry matter yield.

$DSTI_{TDM}$: DSTI computed based on total dry matter yield.

E: Evaporation.

EARO: Ethiopian Agricultural Research Organisation.

ELISA: Enzyme Linked Immuno-sorbent Assay.

Es: Soil evaporation.

ET: Evapo-transpiration.

FC: Field capacity.

GH: Growth form.

GLM: General Linear Model.

ILRI: International Livestock Research Institute.

INL: Inter-node length.

IVDOM: *In vitro* digestibility of organic matter.

K: Potassium.

LAR: Leaf area ratio.

LFL: Leaflet length.

LFNO: Leaflet number per leaf rachis.

LFW: Leaflet width.

LL: Length of leaf rachis.

LMF: Leaf mass fraction.

LP: Leaf percentage.

LYLD: Leaf dry matter yield.

MCA: Methanol-chloroform-ammonia mixture.

Mg: Magnesium.

Mn: Manganese.

N: Nitrogen.

NAR: Nett assimilation rate.

NDF: Neutral detergent fibre.

NDFD%: Neutral detergent fibre digestibility coefficient.

NRC: National Research Council.

OM: Organic matter.

OMD: Organic matter digestibility.

OMI: Organic matter intake.

ox-DABA: 4-N-oxalyl-2-4-diaminobutyric acid.

ox-DAPA: 3-N-oxalyl-2-3-diaminopropionic acid.

P; Phosphorous.

PAW: Plant available water.

PC: Principal components.

PC1, PC2 and PC3: Principal component 1, 2 and 3, respectively.

PCA: Principal component analyses.

PET: Potential evapo-transpiration.

PH: Plant height.

PT: Potential transpiration.

PWP: Permanent wilting point.

RGR: Relative growth rate.

RMF: Root mass fraction.

Rpm: Revolutions per minute.

SAS: Statistical Analyses Systems.

SLA: Specific leaf area.

SLW: Specific leaf weight.

SMF: Stem mass fraction.

ST: Stem thickness.

SYLD: Seed yield.

T: Transpiration.

TDMYLD: Total dry matter yield.

TE: Transpiration efficiency.

$T_{LDM}$: Transpiration efficiency based on nett leaf biomass yield.

$T_{TDM}$: Transpiration efficiency based on nett total biomass yield.

WL: Width of leaf rachis.

WSI: Water stress indices.

$WSI_{ET}$: Water stress index computed based on evapo-transpiration component.

$WSI_{T}$, Water stress index computed based on transpiration component.

WU: Water use.

WUE: Water use efficiency.

$WUE_{LDM}$: Water use efficiency based on nett leaf biomass yield.

$WUE_{TDM}$: Water use efficiency based on nett total biomass yield.

Zn: Zinc.

# CHARACTERIZATION AND EVALUATION OF *INDIGOFERA* SPECIES AS POTENTIAL FORAGE AND COVER CROPS FOR SEMI-ARID AND ARID ECOSYSTEMS

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## ABSTRACT

The potential of *Indigofera* species as forage and/or cover crops for semi-arid and arid environments was investigated in several experiments conducted on the Hatfield Experimental Farm in Pretoria, South Africa. Dormancy associated with hard seededness is the main constraint for uniform germination and large-scale propagation of these species. In this study, pretreatment increased germination in most accessions with scarification being more effective than boiling water treatment in six accessions, but not in the case of *I. vohemarensis* 8730. In five accessions (*I. cryptantha* 7067, *I. brevicalyx* 7517, *I. arrecta* 7524, *I. spicata* 8254 and *I. vohemarensis* 8730), scarification improved the total germination percentage, though it simultaneously resulted in higher seed mortality of *I. brevicalyx* 7517, *I. arrecta* 7524 and *I. vohemarensis* 8730 than in the control. In four accessions (*I. brevicalyx* 7517, *I. arrecta* 7524, *I. vohemarensis* 8730 and

*I. trita* 10297), boiling water treatment improved germination percentage without causing any significant risk of seed mortality in the latter three species.

In a field study, 41 *Indigofera* accessions were characterized in terms of morphological and agronomic parameters, using multivariate techniques to describe their phenotypic variability. Eight morpho-agronomic groups with various potentials were identified along with eight determinant characteristics that can be regarded as the core attributes for future *Indigofera* germplasm characterisation. Further evaluation of promising accessions revealed remarkable differences, both between and within species, in terms of plant height, canopy spread diameter, forage biomass, crude protein content, *in vitro* organic matter digestibility and indospicine level of the forage. These suggest the possibility of directly selecting accessions with forage potential for subsequent evaluation with target animals.

The response of four selected *Indigofera* accessions under simulated moisture deficit stress and non-stress conditions exhibited significant variation. *I. amorphoides* was relatively sensitive while *I. vicioides* was able to maintain growth under water stress conditions, while the response of the two *I. arrecta* accessions were intermediate. The influence of season and species on forage quality was also studied. Spring growth had a significantly higher ($P< 0.05$) CP content than autumn growth in all species. *In vitro* digestibility of dry material also tended to decrease from the spring of 2004 to the autumn of 2004. Higher levels of Ca, P, Mg, Zn and Cu concentration were revealed in the leaf meal of the first harvest than in the re-growth harvest. All of the species had Ca, Mg, Zn

and Mn concentration levels that could support the requirements of ruminants. P and Cu were slightly deficient for some of the species in the autumn suggesting the need to supplement P and Cu from other sources. Compared to *Leucaena* forage, *Indigofera* forage had higher apparent organic matter and dry matter digestibility coefficients and higher crude protein and neutral detergent fibre digestibility coefficients. The difference between *Indigofera* and *Leucaena* forage in terms of DM intake per unit of metabolic body weight (DMI g $BW^{-0.75}$ $day^{-1}$) was not significant (P> 0.05). The digestible organic matter intake (DOMI) and digestible crude protein intake (DCPI) of the sheep on *Indigofera* forage was similar to that of sheep fed *Leucaena*. In this study, lack of differences between *Indigofera* and *Leucaena* forage in terms of DOMI, DCPI and DNDFI means that *Indigofera* forage would likely support similar weight gains as that of *Leucaena*, but lower than that of *M. sativa* forage.

# GENERAL INTRODUCTION

Marginal environments in semi-arid and arid regions of the world are commonly characterized as rangelands (Ash and McIvor 2005). Rangeland provides forage to a large proportion of the ruminants in both tropical and temperate regions of the developed and developing world. In Ethiopia, most of the grazing lands are in the arid, semi-arid and sub-humid zones that cover around 61-65% of the landmass. Of these about 12% are in mixed farming systems while the rest are in nomadic pastoralist and agro-pastorals systems (Alemayehu, 1998; MoA, 2000). A distinguishing feature of these marginal, or rangeland, environments is that rainfall is usually too low and/or too variable for regular cropping and as a result they are largely used for livestock production (Ash and McIvor 2005). The vegetation comprises grasses, shrubs and trees that occur in mixtures that range from open grasslands with little tree or shrub cover, to shrub communities with little herbaceous material, and to savanna woodlands where trees or shrubs form a variable layer over a grassy understorey. In Africa, and particularly in Ethiopia, the semi-arid and arid ecological areas are highly susceptible to increasing human and livestock use. Precipitation is a limiting factor for both pasture and crop production due to the low and often erratic distribution of rainfall. Climatic variability, coupled with increased livestock numbers, is placing great demands on forage species and their environments. A lack of feed resources is one of the major constraints in ruminant livestock production in these ecological areas. The level of animal production that could be achieved in any one environment is generally related to the quantity, quality and continuity of feed availability throughout the year, which, in turn, is related to rainfall, temperature, soil

type and fertility. Therefore, improvement in animal productivity requires improved nutrition. The nutrition should be of adequate supply and quality.

Continuous reliance on imported feed grains, such as maize, by certain tropical countries has not improved their ruminant livestock industry (Abdullah and Rajion 1997). The industry currently depends mainly on grazing from marginal lands and fibrous crop residues as their primary food sources. In coutriesl like Ethiopia, natural pasture provides more than 80% of the livestock feed, and the productivity and forage quality varies with altitude, rainfall, soil and cropping intensitsy (Alemayehu, 2005). Hence, forages (grasses, legumes and tree forages) are the most readily available and inexpensive feed, which ruminants are well adapted to utilize, by virtue of their ability to digest lignocelluloses or fibrous materials. Although they provide valuable nutrients for ruminants, the nutritional value of these tropical feed resources (herbaceous, shrubs and tree legumes) is some times limited due to the presence of anti-nutritional factors. Thus, there is a big challenge of matching available feed supplies with the animal's needs. Furthermore, efficient production from ruminants represents a complex balance between the changing nutrient requirements of the ruminants (e.g. growth, pregnancy, lactation), the requirements of the rumen microbial ecosystem for nutrient input and removal (intake, comminution of particles, absorption) and the changing external supply of herbage nutrients (pasture growth, maturity and senescence) (Hodgson and Illius 1996).

Continuous development of new technologies, amongst which the evaluation of new sources of forage can be mentioned, is necessary to achieve these objectives. In the

tropics diet quality of animals can be improved by supplementing grasses with protein rich forage legumes during the growing season, and fodder trees and shrubs during the dry season. A number of exotic species (*Medicago* spp. *Leucaena* spp., S*tylosanthes* spp., etc) have been successfully introduced and remarkable achievements has been recorded in boosting livestock productivity in various parts of the world using different integrated strategies that best fit the different livestock production systems (Pengelly et al. 2003). However, the introduced herbaceous forage legumes have not had a major impact on cultivated pastures of tropical and subtropical Africa, in particular in the semi-arid and arid zones. Here investment in improved forages is unlikely to take place in pastoral and agro-pastoral systems of Africa (including Ethiopia) where livestock are kept for "wealth" or risk aversion in response to the harsh environments, and where farmers and communities have no capacity to invest in new forages because of their economic circumstances. Further, problems with low soil fertility, specific Rhizobia requirements, poor establishment, poor adaptation to pathogens and pests, climatic limitations, short-lived persistence under grazing and prohibitive costs have all contributed to this general failure (Muir and Maposse 1999; Texas A&M University Experimenatal Station, unpublished report). Thus, there is a need to identify plants that can provide palatable forage on a reliable basis, tolerate herbivory, persist under periodic drought conditions and compete successfully with other plant species, such as annual grasses and forbs.

Fortunately, the native species, which are a key forage resource for arid and semi-arid environments of sub-Saharan Africa, have experienced a long history of use by pastoral livestock, and plants have persisted in the presence of various stress conditions (high

temperature, defoliation, low and erratic moisture, soil infertility, etc). Extremely variable topographical and agro-climatic conditions in Ethiopia have produced several major ecological systems that support large and very diverse genetic resources. The identification and development of locally adapted native herbaceous legumes, which up to now, have received little attention, may provide better germplasm for range reseeding and pasture cultivation in these areas. In this regard, herbaceous legumes such as *Indigofera* can be mentioned as examples of under-utilized resources that have received little attention, as have their effective management options or their efficient production and subsequent utilization. Unfortunately, many of these tropical legumes contain secondary plant compounds, which may diminish their potential value as high quality feeds. There is an increasing awareness that the effect of these compounds on feed quality and animal production requires greater attention. It is also apparent that forage and pasture management practices should play a major role in the alleviation of the feed problem in semi-arid and arid areas. However, environmental and economical sustainability is most likely to be achieved if breeding/selection endeavors to develop efficient water-use varieties/ecotypes are successful. In this PhD research programme, several experiments were conducted on the Hatfield Experimental Farm in Pretoria, South Africa, to expand the limited database available in relation to *Indigofera* species and select potentially suitable accessions for forage production and/or as cover crops, that might be used to augment rangelands and/or to rehabilitate degraded rangelands in limiting environments.

**General objective and outline of the study**

The present research was undertaken with the overall aim of investigating the potential of *Indigofera* species as low input legumes useful as a forage or cover crop in limiting environments, semi-arid and arid areas in particular. To accomplish this, available databases in relation to *Indigofera* species and other indigenous legumes, were reviewed in chapter one. In chapter two, hard seed coat dormancy breaking and germination response of selected *Indigofera* accessions to different pre-planting seed treatment options were investigated. In chapter three, 41 accessions of *Indigofera* species, received from International Livestock Research Institute (ILRI), were characterized in terms of different parameters (morphological and agronomic attributes) to describe their phenotypic variability. Subsequently the suitability of promising accessions with high forage potential were evaluated in chapter four in terms of their biomass yield, nutritive value and anti-nutritional factors based on data generated in both field conditions and laboratory analyses of the forage sample. In chapter five, selected *Indigofera* accessions were examined in a glasshouse study for their variation in terms of biomass accumulation, growth parameters, water use and water use efficiency response when subjected to simulated moisture deficit stress and non-stress conditions. The influence of season and species on forage quality was investigated in chapter six, while in chapter seven the voluntary intake and *in vivo* digestibility of *Indigofera* forage, as compared to *Medicago sativa* and *Leucaena leucocephala*, were evaluated using Merino sheep. Finally, chapter eight presented general conclusions and the implication of the results of this study with recommendation for future research areas to realise the potential of

*Indigofera* species demonstrated in this study as forage and cover crop for semi-arid and arid ecological areas.

# CHAPTER 1

# 1.0 LITERATURE REVIEW

## 1.1. Potential of *Indigofera* species as forage crops

*Indigofera* is a large genus with some 700 species in tropical Africa, Asia, Australia and North and South America. According to Hedberg and Edwards (1989), there are about 78 species that were recorded in Ethiopa. Naturally, the *Indigofera* species are distributed across a wide range of agro-ecological areas, which range from arid to sub-humid conditions and at an altitude of less than 2200 m (Abubeker Hassen 2006, Unpublished data) Many species in Africa and Asia are reported to be useful for forage, green manure or as cover crops (Fröman 1975). Apart from this, a number of *Indigofera* species are known to contain the pigment indigo (Aylward *et al.* 1987), which is already used for commercial dye production. Among those occurring in Ethiopia some of the species recommended for forage production by Fröman (1975) include *I. hirsuta*, *I. pilosa*, *I. schimperi*, (syn. *I. oblongifolia*), *I. spicata* and *I. subulata* (syn. *I. trita*) while species such as *I. hirsuta* and *I trita* were recommended for green manure or as cover crops.

Typical of the Leguminosae, the *Indigoferas* are high in protein, and their ability to tolerate drought, floods and salinity makes them agronomically very desirable (Skerman 1982). For example, the dwarf shrubs such as *Indigofera spinosa* are described as a key element of pastoral subsistence in the arid and semi-arid ecosystems of Northern Kenya (Coughenour *et al*. 1990). Key attributes, which make it a valuable forage species, are its palatability (Coppock *et al.* 1986, 1988), its resistance to herbivory (Bamberg 1986; Mugambi 1989), and its ability to respond to small rainfall events (Coughenour *et al*.

1990). The perennial, deep-rooted growth form would also prove important for soil stabilization in regions where soils are sandy and rainfall levels insufficient for perennial grass growth (<350-400 mm/year) (Coughenour *et al.* 1990). The combinations of traits in some species of the genus *Indigofera* are ideal in the semi-arid and arid environments of Africa where pastoralism is an important subsistence mode and rainfall is erratic.

### 1.2. Limitations of *Indigofera* species as forage crops

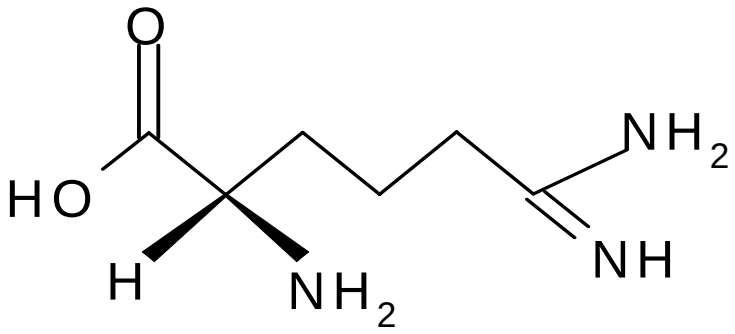
Plants of the genus *Indigofera* have shown great promise as grazing forage and feed supplements for ruminants and non-ruminants. Nevertheless, reservations concerning the toxicity of this genus have restricted its planting (Aylward *et al.* 1987).

#### *1.2.1.* ***Indospicine toxicity***

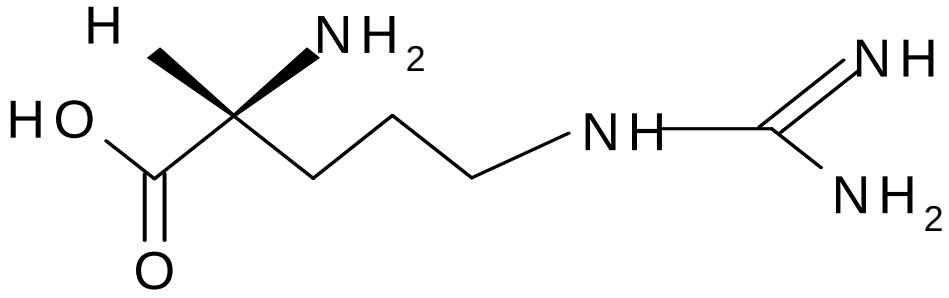
The most studied species, *Indigofera spicata*, has been shown to be toxic to chicks (Britten *et al.* 1963), and to be hepato-toxic when grazed by cattle (Norfeldt *et al.* 1952), or when fed to rabbits (Hutton *et al.* 1958a), mice (Hutton *et al.* 1958b) and rats (Christie *et al.* 1975). A free, non-protein amino acid analog of arginine named 'indospicine' (Hegarty and Pound 1968) was detected in the seed and leaf material of *Indigofera spicata* (Figure 1.1). Among the Ethiopian species, toxicity was also reported in *I. hirsuta*, *I. linifolia* and *I. spicata* (Gillett 1958; Fröman 1975). Very little is known of the palatability and toxicity of other members of the genus (Aylward *et al.* 1987).

According to Strickland *et al.* (1987), 50% of the species in the genera *Indigofera* were either toxic or variably so, with the proportion of palatable species in this genera

averaging 30 %. The same authors reported that, when included as 20 % of a complete diet for experimental rats, the palatability, forage toxicity and feeding value of *Indigofera brevicalyx and Indigofera vicioides* were reported to be similar to those of lucerne




L-Indospicine




L-Arginine

**Figure 1. 1.** Structure of L-Indospicine vs. L-Arginine

(*Medicago sativa*). In contrast, lower palatability and feeding value were reported when compared to lucerne for some of the accessions of *Indigofera spicata and Indigofera trita,* included in the same study (Strickland *et al.* 1987). Information regarding the palatability and toxicity of other species is scanty. Absence of adequate information with regard to the forage potential of other species in the genus, and the observed variability, between and within species, in toxicity and palatability of the genus *Indigofera* indicated the necessity to screen each accession. In this regard, efforts undertaken to exploit this variability in their centre of origin and/or diversity has thus far been inadequate. It is believed that genetic diversity is structured in nature on a massive scale globally, regionally, and locally (Nevo 1988), and ecological heterogeneity plays a predominant role in the differentiation of natural populations of plants and animals (Nevo and Beiles 1989). Particularly in semi-arid and arid environments, heterozygosity and gene diversity were found to be positively, and overall, significantly correlated with rainfall variation and climatic unpredictability (Nevo and Beiles 1988). This condition provides many opportunities for the search for genotypes that possess useful genes for future conservation and sustainable agricultural utilization.

### *1.2.2. Potential toxicity of 3-Nitropropionic acid in Indigofera forage*

In addition to indospicine, for some species of the genus *Indigofera*, 3-nitropropionicacid (3-NPA) has been detected as a toxic ingredient (Aylward *et al.* 1987). Britten *et al.* (1963) reported that chicks are particularly susceptible to pure 3-NPA present in the leaf and stem of *Indigofera spicata*, but are unaffected by the seed, which contains indospicine but no 3-NPA. Strickland *et al*. (1987) found no signs of 3-NPA poisoning,

with no alveolar emphysema or locomotor disturbances (James *et al.* 1980) being observed in any of the experimental rats. It is suggested that plant species that contain less than 2.5% dry matter (DM) of 3-NPA are only marginally toxic (Williams and Davis 1982). The highest 3-NPA reported by Strickland *et al*. (1987) for *Indigofera* species was 0.34% DM, and it is, therefore, unlikely to that any significant 3-NPA toxicity effects with such minimal amounts will be observed. However, Williams (1981) detected 3-NPA in 64 out of 250 species of *Indigofera* in concentrations of 0.5-3% DM and recommended the screening of *Indigofera* species for 3-NPA before use as a forage.

### 1.3. Non-protein free amino acid in forage and range plants

Generally secondary plant compounds that are produced by a large group of plant species (e.g. phenolic compounds and lignin) have received relatively more research attention and have been the subject of a number of reviews (Norton 1994; Kumar and Mello 1995; Lowry *et al.* 1996; Foley *et al.* 1999). However, others, specific to only a certain group of plants, in terms of their occurrence and significance, have received less attention. Non-protein amino acids are among the secondary plant metabolites that cause toxicity in many forage and range plants. Amino acids regularly encountered in living organisms as protein constituents, or as metabolic intermediates, are often named the “common amino acids”, and the remainder, which are much more numerous, but which enjoy a more restricted distribution, as the “uncommon amino acids.” Most uncommon amino acids have been isolated from micro-organisms and plants, though a few are also from the animal kingdom (Bell 1976). In plants, however, the uncommon amino acids, of which 250 have been isolated (Fowden 1974), are usually found in the free state or as simple

condensation products (Bell 1976; Swain 1977). Hence, the name non-protein amino acid is used interchangeably for the free uncommon amino acids in plants. A number of these compounds are intermediates in the synthesis and catabolism of protein amino acids (Lea and Norris 1976).

### *1.3.1.* ***Origin of non-protein free amino acids in plants***

Knowledge with regard to the biosynthetic origin and accumulation in different parts of the plant has significance in understanding the contribution of these compounds to the plants in their specific environments and their subsequent impact on biotic factors. It also helps to identify appropriate strategies useful in minimizing their direct and indirect negative effects on the physiology of animals and humans. Structurally non-protein amino acids can be divided into two categories; those that are close chemical analogues of the 'common' amino acids (e.g. indospicine vs. arginine) and those that are not. Generally the close analogues may arise in three possible ways: they may be formed by the modification of 'common' amino acids; they may arise as a result of modifications to the biosynthetic pathways normally associated with the synthesis of 'common' amino acid; or they may be synthesized by novel routes (Bell 1976).

### ***1.3.2. Site and level of accumulation in plants***

Plants, which synthesise non-protein free amino acids frequently, accumulate them in very high concentrations (Bell 1976). Accordingly, high concentrations of non-protein amino acids are found in seeds of *Grifforia simplicifolia* (14% 5-Hydroxy-L-tryptophan), *Dioclea megacorpa* (7-10% canavanine), and *Mucuna mutisiana* (8% of L-3,4-dihydroxy phenylalanine) (Bell and Janzen 1971, Bell *et al.* 1976). The leaves of the legume

*Leucaena leucocephala* contain 8% mimosine, while the shoots of one *Lilliaceous* species *Convallaria majalis* and the rhizomes of another, *Polygonatum miytiflorum* (Fowden 1959 cited in Bell 1976, Hegarty *et al.* 1964) contain over 3% and 6% of azetidine-2-carboxylic acidic, respectively.

Generalisation of the stage, or stages, at which the non-protein amino acid compounds are synthesized during the life cycles of plants appears to be as difficult as the preferential site of accumulation in plants. For example, canavanine stored in the seeds of *Medicago sativa* disappears rapidly during germination (Bell 1960) while albizine, which is found in high concentrations in the seeds of *Albizia julibrissin*, is also found as a major component of the free amino acid pool in the developing seedlings of this species. Azetidine-2-carboxylic acid, already mentioned as a constituent of *Convallaria* and *Polygonatum*, is also found as the major free amino acid in seeds of the legume *Bussea massaiensus*, while in another legume, *Delonix regia*, this amino acid is absent from the seed but can be detected in the developing seedlings (Bell 1976).

On the other hand, in *Lathyrus sylvestris* L. (flatpea) seeds, 2,4-diaminobutric acid (DABA), a non-protein amino acid, contributed about 10% of the total N and up to 3-4% of the dry weight (Foster 1990). It is present at every developmental stage, and is distributed throughout the plant (Table 2.1). High levels in green forage at the early bud stage are retained in year-old hay in case of DABA (Forster 1988 cited in Foster 1990, Shen *et al.* 1989 cited in Foster 1990). Based on DABA concentrations of flat pea seeds,

**Table 1. 1.** Distribution of non-protein amino acids among tissues of flat pea (*Lathyrus sylvestris* L.) (Source: Foster 1990).

| Plant tissue | Compound[a] | | |
|---|---|---|---|
| | DABA | ox-DABA | ox-DAPA |
| Pericarps | 0.3-5 | 1 | 0.5 |
| Immature seeds | 3 | 1 | 0.5 |
| Mature seeds | 3 | 2 | 2 |
| Leaves | 3.5-19.8 | 1 | 1 |
| Stems | 4-18.7 | 1 | 1.5 |
| Roots | 4-18.7 | 1.5 | 1.5 |
| Flowers | 0-4 | 0.5 | 0.5 |

[a] DABA, 2,4-diaminobutyric acid; ox-DABA, 4-N-oxalyl-2-4-diaminobutyric acid; ox-DAPA= 3-N-oxalyl-2-3-diaminopropionic acid.

[b] Number and symbols reflect relative quantities from very large (5, +++) to trace (0.5).

seedlings, and hay, Foster (1990), concluded that levels of DABA increase markedly at germination where it is highest in seedling tissue, and decreases with age. On the other hand, higher levels of DABA were observed in leaves of flowering plants than in plants at other developmental stages (Foster 1990). Information is not available as to the source-sink relationships during reproductive growth, but the seed straw is suggested to have significantly lower levels of DABA, due to translocation of the compound to the leaves (Foster 1990).

### *1.3.3. Possible role of non-protein amino acid in plants*

Many persons have hypothesized about the production of primary plant metabolites, but little is known as to why plants manufacture secondary metabolites such as non-protein amino acids at all. Therefore, the physiological role of these carbon based nitrogenous toxic compounds in *Indigofera* (indospicine and 3-NPA) is not clearly known. Some scientists (Bell 1976) have argued strongly that " *a plant which diverts as much as 10% of its resources, biosynthetic capacity and storage space to the accumulation of secondary compounds is not going to survive in competition with increasingly less prodigal plants in the same environments unless the presence of these compounds confer some selective advantage on the plant which contains it.*"

One possible advantage, which non-protein free amino acid compounds confer to the plants, is the toxicity of these compounds to biotic factors that may interfere with their establishment, growth and development. Some carbon based secondary compounds, like tannin provide plants with a chemical defence against some mammalian herbivores (Feeney 1976; Bernays *et al.* 1989). Certain woody plants that have evolved under conditions of regular defoliation by animals have developed defence mechanisms to discourage defoliation, in arid environments in particular (Tainton 1999). Such mechanisms may take the form of morphological adaptations (such as thorns or changes in canopy structure) or the accumulation of secondary chemical defense mechanisms. The allocation of energy and nutrients to growth and defence, respectively, is determined by the environmental conditions prevailing at the time of growth (Rhoades 1979). Therefore, stressed plants are able to allocate fewer nutrients to defense than non-stressed plants.

They are consequently more vulnerable to browsing than non-stressed plants (Tainton 1999). Thus, many, if not all, of the non-protein amino acids accumulated in plants are weapons in this chemical armory.

The other reason is that some non-protein amino acid compounds could probably be used as a means of excess nitrogen (N) storage for re-use at a period of critical need by the plant. Levels of DABA tended to increase in *Lathyrus* species throughout the plant when the N was readily available (Table 1.2). Nitrogen fixing plants supplied with inorganic N in the form of nitrate, use DABA as a means to store excess N (Foster 1990). Ammonium N fertilisation was detrimental to the overall health of the flat pea plant, so that accumulation of DABA detected in these plants, when the $NO_3^-/NH_4^+$ ratio was low, may reflect a means by which the plants attempt to relieve the ammonium toxicity.

On the other hand, in other forage legumes, such as alfalfa (*Medicago sativa*), N reserve plays a significant role in relation to defoliation stress tolerance (Sanderson *et al*. 1997), and up to 40 kg/ha of N was remobilised from alfalfa taproots and exported to support aerial re-growth (Lemaire *et al.* 1992). Hendershot and Volenec (1993) determined that specific pools of N in the alfalfa taproot were used for re-growth after cutting. Aspartate and asparagines were the most prevalent amino acids in taproots and along with buffer-soluble proteins decreased greatly in concentration after defoliation. These N compounds

**Table 1. 2.** Changes in 2,4-Diaminobutyric Acid (DABA) levels in flat pea plants exposed to different experimental conditions (Source: Foster 1990).

| Factors | DABA response [a] | | |
|---|---|---|---|
| | Leaves | Stems | Roots |
| Age | + | + | 0 |
| Nitrogen availability | +++ | ++ | ++ |
| NH4+ toxicity | ++ | ++ | ++ |
| Drought | | | |
| Severe, young plants | ++ | + | +++ |
| Mild, old plants | + | + | 0 |

[a] Symbols reflect relative quantities from very large (5, +++) to trace (0.5).

were then replenished during shoot re-growth. The amino-N compounds were postulated to serve as readily available forms of N, whereas the proteins may be a long-term storage form (Sanderson *et al.* 1997). Thus, it is worthy to know the physiological role of these N compounds in relation to the genotypes adaptability to stressed environments and other valuable traits for their subsequent improvement through agronomic and/or breeding manipulations.

Drought stress, too, resulted in an increase in the DABA content that quantitatively far exceeded the amount of prolin in the tissue. This increase, which was expressed primarily in the root, was thought to be too small to provide significant osmotic adjustment under water deficit stress. Each of these stress related changes in DABA levels is superimposed

on increases associated with increasing age of the tissue, nitrogen stored as the diamino acid could conceivably be related to subsequent primary metabolism when the stress is relieved.

### 1.4. Potential management strategies to overcome toxicity in animals

Depending on the nature, path and extent of toxicity caused by non-protein amino acids, specific approaches are adopted successfully for various species to revert or minimize the detrimental effect of these compounds on animals. However, the results are too specific and it shouldn't be adopted before confirming the validity of these approaches for other species, which produce different non-protein amino acid compounds. Some of the success strategies are:

#### 1.4.1. *Screening or development of varieties low in toxic non-protein amino acid*

Mimosine is a non-protein amino acid and an active ingredient responsible for the toxic effect of *Leucaena leucocephala* fed to animals. It was possible to produce cultivars with low mimosine by crossing *Leucaena pulverulenta* and *Leucaena leucocephala* (Jones and Bray 1983). The use of such a strategy, however, requires, as a pre-requisite, a simple and fast method of detecting specific toxic non-protein amino acid compounds in a plant sample. This method permits the determination of the existence of genetic variation in the materials available and also the determination of whether the character is highly heritable or not.

### 1.4.2. *Identification of optimum developmental stage and plant part for utilisation*

Many species that produce non-protein amino acids are toxic under the right set of conditions (Butler and Bailey 1973; Rosental and Jazen 1979), but knowledge of the physiological mechanisms of toxicity and the necessary management procedures to avoid the problem make it possible to use these species as forage. For example, the relative toxicity of *Lathyrus* species forages and seed taken by animals were reported to be different (Foster 1990). The same author reported that intake of *Lathyrus* seeds has most often been associated with illness, while the non-seed parts of flat pea are not lathyrogenic. On the other hand, flat pea hay harvested at the vegetative and early bud stages of growth, did not produce adverse effects when it comprised up to 100% of the ration fed to wether lambs for one month (Forster *et al.* 1986-1987 cited in Foster 1990). In contrast, pelleted flat pea hay, harvested at the pod filling/seed ripening stage of growth, was toxic for both wether lambs and lactating ewes when fed as 70% of the diet (Foster 1990).

Young re-growth of *Indigofera* species has higher N and indospicine than mature growth and stem. Due to its higher indospicine concentration, lower intake and live weight gain were recorded on the younger material of apparently toxic species. Thus, as with N, the concentration of active ingredients (indospicine) varies between the different stages of growth and different plant fractions in *Indigofera* species. For example, seeds of some species have higher toxic content than the forage (Hegarty *et al*. 1979, Strickland 1987). This is because the toxic nitrogenous compounds are mainly synthesized in leaf tissue and translocated, in many instances, later on to the developing seed pod and seed

(Culvenor 1970). This is not true, however, for tannins in some species, and cyanoglycosides and 3-NPA in other, where the toxin occurs in higher concentration in the vegetative material than in the seed. Toxic seeds usually produce their ill effects with lower concentrations of the active ingredients (Johnson 1984; Williams and Daniels 1984), though this varies widely according to the nature of the toxin. This indicates the necessity to identify optimum stage of growth and plant fraction to emphasize if these species are to be promoted for fodder production through range reseeding projects or as a cultivated pasture crop in stressed environments.

### 1.4.3. *Silage production*

Sometimes it is possible that (by virtue of having a specific type of enzyme) a specific type of micro-organisms capable of degrading toxic non-protein amino acids could co-evolve with the plants producing them. Among these, anaerobic micro-organisms, involved in silage production, could conceivably degrade non-protein amino acids, as observed for DABA in flat pea herbage, thereby reducing the levels of the chemical before the silage is presented as a feed for livestock (Foster 1990). In such cases the process could be readily applied to take advantage of the nutritional and economic benefits of silage production and to produce a high quality livestock feed (Foster and Perry 1989 cited in Foster 1990).

### 1.4.4. *Establishing optimum levels of inclusion in the diets of various animals*

By reducing the proportion of toxic plants in the diet, adverse effects can also be reduced. But this is more practical under a “cut and carry system” than under grazing conditions.

The alternative is to use fenced paddocks, or planting of the problematic plants in widely spaced rows, or allowing such plants to grow above grazing height can be adopted to restrict intake to below the threshold levels under grazing (Wildin 1985).

### 1.4.5. *Fertilizer application*

In some species reduction in the level of non-protein amino acid may be achieved by improving the soil nutrient status. The hypothesis is based partly on the observed relationship between deficiencies of some nutrients (e.g. N, K, B, etc.) and the levels of free amino acid concentration in a plant. However, there has been little work done on either supplementation or fertilization as a strategy to alleviate the perceived problems from non-protein amino acid of the various species when used as a forage plant.

### 1.4.6. *Identification of rumen microbes able to detoxify the non-protein amino acid*

As in the case of those anaerobic micro-organisms involved in silage production, some specific rumen micro-organisms may metabolise some of the toxic non-protein amino acid. Particularly in the centre of origin and diversity of the plant rumen microbes capable of degrading toxic non-protein amino acid compounds of a specific plant might co-evolve, to subsequently result in improved utilisation of plants with potentially toxic non-protein amino acid. The rumen microbes could metabolise such compounds in several ways: they may convert the toxin to non-toxic metabolites; they may convert the toxin to compounds with enhanced activity in the animal; they may convert the toxin to substances with a completely different toxic property; or they may not metabolise the toxin at all, although subsequently some change may occur in the body tissue.

Besides with an increasing understanding of the structure of non-protein amino acid produced by the various plants and their likely degradation pathways, it may be possible to modify bacteria genetically to contain specific enzymes that detoxify the problematic free amino acid compounds. In this regard the successful use of naturally occurring DHP degrading bacteria to solve the *Leucaena* toxicity problem in Australia offers hope and encouragement to search for other microbes capable of detoxifying specific free amino acids, which limit the use of species that have superior traits, but not in terms of their quality (Jones and Megarrity 1986).

### 1.5. Conclusion and hypothesis formulation

Generally, regardless of proven adaptability of *Indigofera* species to the semi-arid and arid ecological areas of sub-Saharan Africa (including Ethiopia), the majority of the species in the genus *Indigofera* are under-utilized and often under-represented in studies of forage resources. It is true that the lack of adequate scientific information and understanding with regard to the resource base, ecology and genotypic variation of superior agronomic and nutritive value traits has limited the potential use of these species as feed and fodder legumes. Besides, the presence of non-protein amino acids in the forage imposes a threat for the optimum utilisation of plants, which produce indospicine compounds. Due to this, little attention was given to these plants for use as forage or cover crops, in the past. However, more recently in America there is a shift in philosophical focus towards conservation and restoration of “natural” communities instead of maximizing productivity (Anderson 2003).

In recent years, however, livestock have been pushed into marginal areas and overgrazing and its associated effects (e.g. land degradation, soil erosion, low nett primary productivity, etc.) are not uncommon in communal grazing areas. Here the dominant species are characterised by the presence of secondary compounds including non-protein free amino acids. Such plants are also a major component of early- and mid-successional stages in grassland ecosystems. The presence of non-protein free amino acid might have conferred a competitive advantage over other species in such communities. However, the exploitation of these resources for animal production purposes pre-supposes a proper understanding of the biosynthetic origin, preferential site of accumulation, possible role in plants, their impact on biotic factors and their mode of interference in grazing animals. Although, for some species, potential management strategies are explored with the aim to avoid or minimize their detrimental effects (e.g mimosine in *Leucaena* spp.), the result is often too specific, depending on the type of specific non-protein amino acid compounds involved. Hence, there is a need to develop and refine appropriate management procedures for optimal utilisation of each species having a specific non-protein amino acid if these plants are to be promoted as new forage or cover crop.

In this study, it is hypothesized that promoting indigenous species has the advantage over exotic species in limiting environments, due to the fact that the species, or genotypes, are already well adapted to their particular habitats. This is because many of the indigenous species have colonized areas with different climatic, biotic and abiotic conditions during their growing season through differentiated ability to tolerate various stresses, which would imply the possibility of generating cultivars of *Indigofera* that might be used either

to augment native rangelands and/or that might be sown in degraded rangelands in semi-arid and arid areas of Ethiopia. It is, therefore, necessary to study some of these species and/or accessions for their morphological, agronomic, nutritive value and anti-quality trait variation and evaluate selected accessions for their tolerance to water deficit stress, a common phenomenon in semi-arid and arid areas, to identify accessions with high potential as forage and cover crops.

### 1.6. Specific objectives

This PhD thesis research has focused on *Indigofera* species among the many potential candidate species with the following specific objectives:

- To identify appropriate pre-planting seed treatments that will maximise germination and improve subsequent establishment. It is expected that the accessions will respond variably to the various treatment options.

- To characterize and study variation between accessions of *Indigofera* species in terms of morphological and agronomic traits. It is expected that these characters differ both between and within a species due to variation in collection site ecological heterogeneity, which plays a predominant role in the differentiation of natural populations.

- To select superior accessions of *Indigofera* species in terms of high forage yield, high nutritive value and low phyto-toxin content, when grown under field

condition at Pretoria for immediate (improve range land productivity) and long term (prioritise conservation of other genotypes with valuable traits) utilisation. It is expected that differences between accessions will occur and genetically superior characters will maintain their stability in a range of environments.

- To investigate the influence of a range of moisture conditions on growth, forage yield, water use and water use efficiency of selected accessions of *Indigofera* species. It is expected that differences between species responses in terms of studied parameters, will occur due to the variation in the influence of different moisture levels.

- To investigate the influence of season and species on forage quality of selected *Indigofera* species. It is expected that differences between species responses in terms of forage quality parameters, will occur due to temporal variation in the climatic conditions of the growing environment.

- To determine the *in vivo* digestibility and forage intake of *Indigofera* species as compared to lucerne and *Leucaena* forage by Merino sheep. It is expected that there will be variation in terms of digestibility and subsequently intake and thus ranking of the feed due to variation in chemical composition and anti-quality factors of the three forages.

# CHAPTER 2

# Effect of pre-planting seed treatment on dormancy breaking and germination of *Indigofera* accessions

## 2.1. Abstract

A factorial treatment combination of seven different accessions of *Indigofera* (*I. cryptantha* 7067, *I. brevicalyx* 7517, *I. arrecta* 7524, *I. spicata* 8254, *I. vohemarensis* 8730, *I. trita* 10297 and *I. spicata* 10299) and three different seed pretreatments (untreated or control, scarified and immersed in boiling water at 98ºC) were evaluated. Pretreatment increased germination in most accessions with scarification being more effective than the boiling water treatment in six accessions, but not in the case of *I. vohemarensis* 8730. In five accessions (*I. cryptantha* 7067, *I. brevicalyx* 7517, *I. arrecta* 7524, *I. spicata* 8254 and *I. vohemarensis* 8730), scarification improved the total germination percentage, though it simultaneously resulted in higher seed mortality of *I. brevicalyx* 7517, *I. arrecta* 7524 and *I. vohemarensis* 8730 than in the control. In four accessions (*I. brevicalyx* 7517, *I. arrecta* 7524, *I. vohemarensis* 8730 and *I. trita* 10297), boiling water treatment improved germination percentage without causing any significant risk of seed mortality in the latter three species.

**Key words:** Dormancy breaking, hardseed, seed germination, seed mortality, seed treatment.

## 2.2. Introduction

*Indigofera* species show great promise as grazing forages for ruminants. Typical of Leguminosae, *Indigofera* species are high in protein. Their ability to tolerate drought, floods and salinity makes them agronomically desirable (Skerman 1982). Their deep-rooted growth form, ability to respond to small rainfall events and resistance to herbivory make them potentially valuable cover crops and forage species for semi-arid and arid areas. Strickland *et al*. (1987) report that about 50% of the species in the genus are toxic to some degree, but only 30% are palatable. The forage toxicity and feeding value of *Indigofera brevicalyx* and *I. vicioides* have been reported to be similar to that of *Medicago sativa* (lucerne), while higher toxicity and lower feeding values have been reported for *I. spicata* (Strickland *et al.* 1987).

Germination, emergence and establishment of legumes depend on the interaction of biological, environmental and management variables. In semi-arid and arid conditions, which prevail in parts of Ethiopia, seedling emergence and establishment are constrained mainly by the irregular distribution of rainfall within a season. Apart from this, seed size, weight, dormancy and integument thickness have significant effects on the emergence and establishment of seedlings from soil seed banks under natural conditions (Carren *et al*. 1987; Veenendaal *et al*. 1996; Sy *et al*. 2001). The extent of seed dormancy needs to be within acceptable levels for range reseeding projects to be profitable, while uniform germination is probably more beneficial in the case of sown pastures.

Poor germination was experienced in more than 50% of *Indigofera* accessions received from the International Livestock Research Institute (ILRI) gene bank for a characterisation study being conducted in Pretoria. The major cause was dormancy associated with hard seed (Abubeker, unpublished data). Although different pre-planting treatments are reported to be effective for breaking hard seed dormancy in different legume species (Hanna 1973; Grant 1979; Dell 1980; Buttler *et al.* 1982; Ramamoorthy and Rai 1990), little has been documented in the case of *Indigofera* species.

From the accessions which exhibited a poor germination rate, six species were selected at random and were included with an accession with an acceptable level of germination in the present study. The aim of the study was to compare the suitability of pre-planting seed scarification and treatment with boiling water as practical techniques to break seed dormancy and enhance germination of different *Indigofera* species.

**2.3. Material and methods**

The seeds of the *Indigofera* species studied were received from the ILRI gene bank and were collected from forage seed production sites of ILRI at Zeway and Soddo, in Ethiopia. The six species known to have poor germination were: *I. cryptantha* 7067, *I. brevicalyx* 7517, *I. arrecta* 7524, *I. spicata* 8254, *I. vohemarensis* 8730 and *I. trita* 10297, while *I. spicata* 10299 had reasonable germination. A factorial combination of these seven accessions and three seed pretreatments (untreated or control, seed scarified and seed treated with boiling water) were evaluated in a completely randomised design with three replications.

About two g of seed from each accession were subjected to either mechanical scarification (rubbing the seeds between sand paper) or boiling water treatment (placing seed in boiling water and leaving until the water cooled). At Pretoria, water boils at 98 °C because of the altitude (1350 m asl). After treatment, 50 seeds from each treatment were placed in petri dishes fitted with moist filter paper. These were placed in a growth cabinet set to 12 hours light/12 hours dark and day/night temperature of 30/20 °C. Seeds were adequately watered throughout the experimental period with distilled water. Germination counts were made every three days for 15 days. Seeds were considered germinated when the radicle had emerged through the integument; germinated seeds were removed after each count. At the end of the test, seeds that had not germinated were categorised into hard and dead components by touching and piercing with a needle. While dead seeds could be pierced with the needle, hard seeds could not.

The percentages of germinated, hard and dead seeds were subjected, after arcsine transformation, to analysis of variance using Proc GLM of SAS (2001). When Fisher's F values were significant at $P<0.05$, the analysis was continued by comparing the means using Tukey's test at $P<0.05$. Arcsine-transformed means were back-transformed for presentation.

**2.4. Results and discussion**

There was a significant ($P<0.05$) interaction between *Indigofera* accession and effect of seed treatment, suggesting that any effects of treatment on dormancy breaking,

germination rate or seed mortality should be assessed separately for each accession. Hence, data for individual species for each treatment are presented.

### 2.4.1. *Hard seed breakdown*

The accessions known to have low germination rates showed 75 – 95 % hard seed after the two-week germination test in untreated controls. This high proportion of hard seed is similar to that reported in an earlier pilot study (Abubeker, unpublished data). Hard seededness is a well known phenomenon in many leguminous species (Skerman 1982) *e.g. Cassia obtusifolia* (Sy *et al*. 2001) and *Acacia senegal* (Danthu *et al*. 1992). It is an important trait that enhances the chance of survival of a species by ensuring sequential germination of seed from the soil seed bank in arid and semi-arid areas, where the climate is often extreme and highly variable with erratic starts to the wet season. From the perspective of introducing a legume into a sown pasture, reseeding rangeland or pasture renovation, a high proportion of hard seed in a seed lot could, however, have a negative impact on rapid establishment.

In all accessions, the percentage of hard seed remaining at the end of the germination test was significantly higher ($P<0.05$) in the control seeds than in those either scarified or treated with boiling water (Table 2.1). Both types of treatment obviously damaged the seed integument allowing the penetration of water, and increasing the level of germination, as demonstrated by Elberse and Breman (1989).

**Table 2. 1.** Effect of scarification and treatment with boiling water on percentage of hard seed after incubation in a growth cabinet for two weeks.

| Species/accessions | Percentage of hard seed | | |
|---|---|---|---|
| | Untreated seed (control) | Seed pretreatment | |
| | | Scarification[1] | Boiled water[2] |
| *I. cryptantha* 7067 | 88 Auv | 0 Cx | 72 By |
| *I. brevicalyx* 7517 | 95 Du | 0 Fx | 11 Ea |
| *I. arrecta* 7524 | 75 Gr | 0 Ix | 28 Hz |
| *I. spicata* 8254 | 85 Juv | 1 Lx | 60 Ky |
| *I. vohemarensis* 8730 | 95 My | 0 Nx | 0 Nb |
| *I. trita* 10297 | 79 Ov | 0 Qx | 7 Pa |
| *I. spicata* 10299 | 55 Rw | 0 Tx | 17 Sza |

[1] Seed rubbed with sand paper.

[2] Seed immersed in boiling water and left until the water cooled down.

[3] Means within rows followed by the same uppercase letter or within columns followed by the same lower case letter are not significantly different (P>0.05).

Scarification broke hard seed dormancy to a significantly ($P<0.05$) greater extent than boiling water treatment in all accessions, except *I. vohemarensis* 8730. Scarification would fracture the seed testa in many places and allow rapid imbibition of water, while the boiling water treatment would rupture the seed coat by ejecting the strophiolar plug and cracking the testa (Argel and Paton 1999). In the case of the boiling water treatment, water imbibition would occur over a relatively longer period of time than with the fractured seed testa from scarification.

### 2.4.2. *Germination and mortality*

The total percentage germination and mortality of seeds from the different treatments and accessions are presented in Tables 2.2 and 2.3, respectively. In five accessions (*I. cryptantha* 7067, *I. brevicalyx* 7517, *I. arrecta* 7524, *I. spicata* 8254 and *I. vohemarensis* 8730), while scarification significantly ($P<0.05$) increased the total germination percentage compared with the control, it simultaneously and significantly ($P<0.05$) increased the level of seed mortality of *I. brevicalyx* 7517, *I. arrecta* 7524 and *I. vohemarensis* 8730 accessions relative to the control. This agrees with the results of Hopkinson and Paton (1993), who reported increased laboratory germination of *Stylosanthes scabra* cv. Seca seed with a slightly increased risk of causing seed death.

In contrast, boiling water treatment significantly ($P<0.05$) increased germination in four accessions (*I. brevicalyx* 7517, *I. arrecta* 7524, *I. vohemarensis* 8730 and *I. trita* 10297), but seed mortality was increased in only a single accession, *I. brevicalyx* 7517. Phipps (1973) reported similar increases in germination in *Centrosema pubescens* seed following

**Table 2. 2.** Effect of scarification and treatment with boiling water on percentage of seeds which germinated in a growth cabinet over two weeks.

| Species/accessions | Percentage of germinating seed | | |
|---|---|---|---|
| | Untreated seed (control) | Seed pretreatment | |
| | | Scarification[1] | Boiling water[2] |
| *I. cryptantha* 7067 | 3 Ap | 73 Br | 11 Aw |
| *I. brevicalyx* 7517 | 2 Cp | 41 Dr | 47 Duv |
| *I. arrecta* 7524 | 10 Epq | 49 Fr | 57 Ftuv |
| *I. spicata* 8254 | 11 Gpq | 73 Hr | 26 Gvw |
| *I. vohemarensis* 8730 | 2 Ip | 56 Jr | 89 Kt |
| *I. trita* 10297 | 15 Lpq | 46 LMr | 73 Mtu |
| *I. spicata* 10299 | 31 Oq | 53 Or | 43 Ouv |

[1] Seed rubbed with sand paper.

[2] Seed immersed in boiling water and left until the water-cooled down.

[3] Means within rows followed by the same uppercase letter or within columns followed by the same lower case letter are not significantly different (P>0.05).

**Table 2. 3.** Effect of scarification and treatment with boiling water on percentage of dead seeds remaining after incubation in a growth cabinet for two weeks.

| Species/accessions | Percentage of dead seed | | |
|---|---|---|---|
| | Untreated seed (control) | Seed treatment | |
| | | Scarification[1] | Boiling water[2] |
| *I. cryptantha* 7067 | 9 Al | 27 Am | 17 An |
| *I. brevicalyx* 7517 | 3 Bl | 59 Cm | 43 Cn |
| *I. arrecta* 7524 | 15 Dl | 51 Em | 15 Dn |
| *I. spicata* 8254 | 5 Fl | 26 Fm | 14 Fn |
| *I. vohemarensis* 8730 | 3 Gl | 44 Hm | 11 Gn |
| *I. trita* 10297 | 6 Il | 54 Jm | 21 IJn |
| *I. spicata* 10299 | 15 Kl | 47 Km | 39 Kn |

[1] Seed rubbed with sand paper.

[2] Seed immersed in boiling water and left until the water-cooled down.

[3] Means within rows followed by the same uppercase letter or within columns followed by the same lower case letter are not significantly different (P>0.05).

immersion in boiling water for a period of one second to 20 minutes or leaving it to cool down. Hopkinson and Paton (1993) studied the effects of immersion in boiling water on germination of *Desmanthus* seed and found that immersion of high quality seed in boiling water for brief periods (4-10 seconds) consistently softened a high proportion of seed without causing serious mortality. Extending the period of immersion led to a progressive increase in the proportion of seed deaths.

**2.5. Conclusion**

As a practical technique to overcome poor germination, associated mainly with hard seed dormancy, the present study found considerable variation among the accessions in terms of their response to pre-planting treatment of seed. An effective treatment method should significantly improve germination rate of the seed lots without causing a significant increase in the mortality of potentially viable seeds. This has been successfully achieved in *I. cryptantha* 7067 and *I. spicata* 8254 by scarification. In contrast, improved germination rates of *I. vohemarensis* 8730, *I. arrecta* 7524 and *I. trita* 10297 were obtained, without significant seed mortality, by immersion in boiling water. The effects of the two treatment methods are similar for both *I. brevicalyx* 7517 and *I. spicata 10299.* While either technique can be used to increase germination in the case of *I. brevicalyx* 7517, significant seed mortality may result. With *I. spicata* 10299, which has a lower proportion of hard seed (54 %) than other accessions (>75%), both techniques will give some improvement in germination but seed mortality can be 40 – 50 %.

Previous studies with *Leucaena leucocephala* have reported that manipulation of hot water temperature is more effective than immersion time in breaking hard seededness while minimising seed mortality (Oakes 1984). Further improvements in germination could be expected in the case of *I. brevicalyx* 7517 by determining optimum hot water temperature below 98°C (the boiling point of water at Pretoria) and/or identification of optimum immersion time.

# CHAPTER 3

# Morphological and agronomic characterisation of *Indigofera* species using multivariate analysis

## 3.1. Abstract

Knowledge of the existing genetic variation between various morphological and agronomic traits is vital for any collection, conservation and breeding programmes. Forty-one *Indigofera* accessions from eight different species were studied in a randomised block design with three replicates, to characterise the accessions, using morphological and/or agronomic data analysed using multivariate methods to identify a core set of attributes to be used in characterisation of *Indigofera* germplasm. Morphological data were obtained from nine plants in each accession while 15 plants were harvested in each accession for dry matter yield determinations. Principal component analyses indicated that the first two components accounted for 80.0, 92.5 and 73.9% of the total variability for morphological, agronomic and combined data sets, respectively. Cluster analysis, using morphological data, revealed six main groups, with *I. coerulea* 9004 being classified in a separate group due to its large stem diameter, leaf and leaflet size (length and width). Five main agronomic groups were highlighted in cluster analyses of the agronomic data. Nine accessions were included in the agronomic group II and III characterised by tall plants with low leaf percentages. Among the rest, *I. vicioides* 10486 was classified in a separate group due to its high leaf yield. Clustering of combined morphological and agronomic data revealed eight main groups. Once again two high yielding groups (IV and V) were identified on the basis of their plant height, stem yield, total dry matter yield and canopy diameter. A character discard resulted in the selection of eight determinant characteristics, namely: growth habit, days to 50% flowering, extent of branching, leaflet length, leaf yield, plant height or length of the principal stem, leaf percentage and canopy spread measured at the widest point. These can be regarded as the core attributes for *Indigofera* germplasm characterisation, which can be used for the identification of suitable breeding material for specific purposes.

**Keywords:** Agronomic traits, characterisation, evaluation, morphological traits, multivariate technique.

### 3.2 Introduction

*Indigofera* species have great promise as forages for ruminants. Their high protein levels and ability to tolerate drought, floods and salinity make them agronomically desirable (Skerman 1982), while their deep-rooted growth form, ability to respond to small rainfall events and resistance to herbivory make them potentially valuable cover crops and forage species for semi-arid and arid areas. According to Strickland *et al.* (1987), however, about 50% of the species in the genera are toxic to some degree and only 30% are palatable. The palatable species have great potential as forages (Fröman 1975), while the unpalatable species are probably best suited as cover crops, especially in limiting environments (*e.g.* dry, arid and desert ecological areas) and degraded rangelands, where insect pests and wild herbivores often militate against the establishment and growth of such cover crops.

The International Livestock Research Institute (ILRI) has collected *Indigofera* accessions from several locations in Ethiopia and maintains them in its gene bank. Considerable morphological variation exists within the genus (Hedberg and Edwards, 1989). A few accessions were originally obtained from Centro Internacional de Agricultura Tropical (CIAT) and these were included in a characterisation study of 41 accessions from 8 species in Pretoria (South Africa). In the study, the morphological and agronomic characteristics were described and characteristics identified which could be used to distinguish between, or group, similar accessions. The analysis of traits that contribute to the genetic variability could help identify selection criteria to improve the productivity and quality of such forage crops.

### 3.3 Materials and methods

The trial was carried out on the Hatfield Experimental Farm, University of Pretoria (1370 m asl). Seeds of 41 *Indigofera* accessions were sown in trays in a nursery (Table 3.1). After establishment, 54 seedlings of each accession were transplanted into field plots in January 2003. Eighteen seedlings were planted as spaced plants in a 1.5 m x 3 m plot area with a spacing of 50 cm between rows and plants, and each accession was replicated three times. Plots were arranged parallel to each other along their length and turned once within a block to minimise variation. Spacings of 50 and 100 cm were maintained between adjacent plots and blocks, respectively. The plants were irrigated twice per week for two hours depending on rainfall events. Plots were kept weed-free by hand pulling. Twenty-one characteristics were observed in each plot (Table 3.2). A total of 9 plants (first plant of each row with a total of 3 plants per plot per rep) of each accession were observed for morphological and some agronomic parameters, while 15 plants of each accession (5 middle plants, including 1 border plant, per plot per replication) were considered for estimation of dry matter yields with harvestable plot area of 1.25 $m^2$. Plants were harvested at the 50% flowering stage to a height of 10 cm for prostrate forms and 15 cm for erect forms, and separated into leaf and stem components, which were dried in a forced-draft oven at 70 °C for 48 hours.

All data were subjected to multivariate analytical methods to explore natural groupings in the data, and to investigate variations between and within groups of accessions. Correlations between characteristics were computed on the mean values of the accession. All variables were standardised to a mean of 0 and a variance of 1 and used in a principal

**Table 3. 1.** Origin of *Indigofera* accessions used in the trial.

| No | Species | ILRI No. | Country of origin | Longitude | Latitude | Elev. (m) | Rainfall (mm) | Mean temp. (°C) | Soil pH |
|---|---|---|---|---|---|---|---|---|---|
| 1 | *I. amorphoides* | 7521 | Ethiopia | 8.50 N | 40.01 E | 1000 | 700 | 26 | |
| 2 | *I. amorphoides* | 7549 | Ethiopia | 8.46 N | 39.37 E | 1150 | 940 | 24 | 8 |
| 3 | *I. amorphoides* | 7557 | Ethiopia | 8.51 N | 39.45 E | 1100 | 700 | 24 | 8 |
| 4 | *I. amorphoides* | 7570 | Ethiopia | 9.00 N | 39.50 E | 1000 | 500 | 26 | |
| 5 | *I. amorphoides*[1] | 7069 | | | | | | | |
| 6 | *I. arrecta* | 7524 | Ethiopia | 7.50 N | 38.40 E | 1700 | 600 | 21 | 6 |
| 7 | *I. arrecta* | 7592 | Ethiopia | 7.48 N | 38.38 E | 1700 | | | |
| 8 | *I. arrecta* | 7598 | Ethiopia | 7.48 N | 38.38 E | 1700 | | | |
| 9 | *I. arrecta* | 7709 | Ethiopia | 10.18 N | 37.50 E | 2470 | 1200 | 16 | 5 |
| 10 | *I. arrecta* | 8644 | Ethiopia | 6.53 N | 38.20 E | 1880 | 1100 | 19 | 6 |
| 11 | *I. arrecta* | 9045 | Ethiopia | 7.05 N | 38.30 E | 1680 | 970 | 18 | 7 |
| 12 | *I. arrecta* | 10339 | Ethiopia | 6.10 N | 37.35 E | 2260 | 1500 | 17 | 5 |
| 13 | *I. arrecta* | 10350 | Ethiopia | 6.09 N | 37.35 E | 1880 | 1140 | 19 | 7 |
| 14 | *I. arrecta* | 10355 | Ethiopia | 6.00 N | 37.33 E | 1400 | 900 | 22 | 8 |
| 15 | *I. arrecta* | 10478 | Ethiopia | 6.50 N | 37.45 E | 1925 | 1300 | 19 | 7 |
| 16 | *I. arrecta* | 10479 | Ethiopia | 6.50 N | 37.45 E | 1925 | 1300 | 19 | 6 |
| 17 | *I. arrecta*[1] | 7850 | Ethiopia | 11.26 N | 39.38 E | 2610 | 1200 | 15 | 8 |
| 18 | *I. brevicalyx* | 7815 | Ethiopia | 11.49 N | 39.34 E | 2900 | 1000 | 13 | 8 |
| 19 | *I. brevicalyx*[1] | 7848 | Ethiopia | 11.26 N | 39.38 E | 2601 | 1200 | 15 | 8 |
| 20 | *I. coerulea* | 9004 | Ethiopia | 9.00 N | 40.04 E | 1000 | 500 | 24 | |
| 21 | *I. costata* | 8712 | Ethiopia | 6.10 N | 37.10 E | 1235 | 900 | 23 | 7 |
| 22 | *I. cryptantha* | 7067 | CIAT | | | | | | |
| 23 | *I. cryptantha* | 7070 | | | | | | | |
| 24 | *I. spicata* | 7682 | Ethiopia | 10.21 N | 38.09 E | 2410 | 1200 | 16 | 7 |
| 25 | *I. spicata* | 8254 | Ethiopia | 7.01 N | 39.03 E | 2370 | 1000 | 16 | 7 |
| 26 | *I. spicata* | 8282 | Ethiopia | 7.12 N | 38.36 E | 1850 | 1200 | 19 | 6 |
| 27 | *I. spicata* | 8290 | Ethiopia | 7.27 N | 38.41 E | 1680 | 700 | | 7 |
| 28 | *I. spicata* | 8301 | Ethiopia | 7.54 N | 38.43 E | 1540 | 700 | 21 | 7 |
| 29 | *I. spicata* | 8305 | Ethiopia | 8.09 N | 38.48 E | 1600 | 700 | 21 | 8 |
| 30 | *I. spicata* | 8312 | Ethiopia | 8.32 N | 39.12 E | 1600 | 800 | 21 | 6 |
| 31 | *I. spicata* | 8413 | Ethiopia | 8.43 N | 36.28 E | 2050 | 1900 | 16 | 5 |
| 32 | *I. spicata* | 8726 | Ethiopia | 6.19 N | 36.55 E | 1375 | 1500 | 22 | 6 |
| 33 | *I. spicata* | 10278 | Ethiopia | 6.44 N | 37.39 E | 1780 | 1300 | 20 | |
| 34 | *I. spicata* | 10408 | Ethiopia | 6.49 N | 37.46 E | 1900 | 1300 | 19 | 5 |
| 35 | *I. spicata* | 10442 | Ethiopia | 6.49 N | 37.46 E | 1925 | 1300 | 19 | 6 |
| 36 | *I. spicata* | 10473 | Ethiopia | 6.45 N | 37.44 E | 1800 | 1300 | 19 | 6 |
| 37 | *I. spicata* | 10504 | Ethiopia | 6.50 N | 37.43 E | 1800 | 1200 | 20 | 7 |
| 38 | *I. spicata* | 10522 | Ethiopia | 6.50 N | 37.46 E | 1925 | 1300 | 19 | 6 |
| 39 | *I. spicata* | 13650 | Ethiopia | | | 1580 | | | |
| 40 | *I. trita*[1] | 9795 | Ethiopia | 5.47 N | 39.17 E | 1760 | 900 | 20 | 5 |
| 41 | *I. vicioides* | 10486 | Ethiopia | 6.50 N | 37.45 E | 1925 | 1300 | 19 | 6 |

[1]Accessions, for which passport data do not agree with the species classification and observed morphological characteristics, have been renamed, with their proper species names, but with the same source accession number.

**Table 3. 2.** List of characteristics observed.

| Abbreviation | Characteristic | Definition | No of plants observed |
|---|---|---|---|
| **Agronomic characters** | | | |
| PH | Plant height or Stem length | Average height of the plants at 50 % flowering for erect and semi-erect accessions or length of the main culms in prostrate plants (cm) | 9 |
| TDMYLD | Total DM yield | Dry weight of above-ground biomass harvested at 50% flowering stage to a height of 10-15cm (g/harvestable plot area[1]) | 15 |
| SYLD | Stem DM yield | Dry weight of stem biomass measured at 50% flowering stage (g/ harvestable plot area[1]) | 15 |
| LYLD | Leaf DM Yield | Dry weight of leaf biomass measured at 50% flowering stage (g/harvestable plot area[1]) | 15 |
| LP | Leaf percentage | Dry leaf biomass as a percentage of total dry above-ground biomass | 15 |
| CSDm | Canopy diameter (maximum width) | Diameter of plant canopy spread measured at widest point (cm) | 9 |
| CSDr | Canopy diameter (width at right angle to the maximum) | Diameter of plant canopy spread measured at right angle to the maximum/widest point (cm) | 9 |
| CSDav | Mean canopy diameter | Estimate of two horizontal diameter of plant canopy spread measured at widest point and right angle to the widest (mean of 2 perpendicular measurements per plant) (cm) | 9 |
| **Phenological and morphological characters** | | | |
| GH | Growth form | Average angle of stem growth direction to the ground (1-9 scale; 1-3 prostrate, 4-6 semi-erect and 7-9 erect) | 9 |
| DFF | Days to first flowering | Number of days taken from planting to appearance of the first flower | Full plot |
| DFPF | Days to 50% flowering | Number of days taken from planting to appearance of flowers on 50 % of the plants | Full plot |
| LL | Length of leaf rachis | Length of leaf rachis including petal and terminal leaflet measured at the $4^{th}$ or $5^{th}$ unfolded leaf from the tip of the main stem (mm) | 9 |
| WL | Width of leaf rachis | Width of leaf rachis measured at widest point on the $4^{th}$ or $5^{th}$ unfolded leaf from the tip of the main stem (mm) | 9 |
| LFNO | Leaflet number per leaf rachis | Average number of leaflets on the $4^{th}$ or $5^{th}$ unfolded leaf rachis | 9 |
| LFL | Leaflet length | Length of the middle leaflet (leaf blade) including petioles taken from the $4^{th}$ or $5^{th}$ unfolded leaf rachis (mm) | 9 |
| LFW | Leaflet width | Width of the middle leaflet at widest point taken from the $4^{th}$ or $5^{th}$ unfolded leaf rachis (mm) | 9 |
| WLR | Width:length ratio of leaflets | Leaflet width as a proportion of its length computed from the leaflet measurements | 9 |
| LFSH | Leaflet shape | Shape of leaflets (leaf blades) (3-7 scale: 3 elongated, 5 intermediate and 7 rounded) | 9 |
| BS | Branching score | A visual assessment of the extent of primary, secondary and tertiary branching of the stem (1-9 scale: 1-3 poor, 4-6 medium and 7-9 high) | 9 |
| ST | Stem thickness | Stem diameter measured half way between nodes for the $4^{th}$ and $5^{th}$ unfolded leaves from the apex of the main stem (mm) | 9 |
| INL | Inter-node length | Length of the inter-node measured from the $4^{th}$ to the $5^{th}$ leaf rachis (mm) | 9 |

[1]Harvestable plot area= 1.25 $m^2$.

component analysis. Where pairs of variables had a correlation coefficient greater than 0.8, one of these variables was omitted to avoid indirect weighting in cluster analysis. This was employed for the separate cluster analyses for morphological and agronomic data sets.

Characteristics were discarded, as proposed by Jolliffe (1972; 1973), to identify a core set of attributes and to reduce the number of characters to be utilised in the combined analysis of morphological and agronomic data sets to formulate a new principal component and clustering analysis. Hierarchical clusters were formed using unweighted pair-group average linkage algorithms of NCSS (Jerry 2000) statistical packages. Variations between the main groups of accessions for the different characteristics were assessed by one-way analyses of variance using SAS (2001), considering groups as treatments and individual accessions within a group as replications.

## 3.4. Results

### *3.4.1* ***Analysis of morphological characteristics***

Separate principal component (PC) analysis for the morphological data set revealed three components with Eigenvalues greater than 1 (Table 3.3). The first 2 principal components (PC) explained 80.0% of the total variation. In particular, the first principal component (PC1), which explained 48.3% of the total variation, was positively associated with growth habit, leaflet number, days to first flowering and days to 50% flowering, while it was negatively associated with leaflet width:length ratio, internodal length and leaflet shape. The second PC (PC2), which explained 32.1% of the total variation, was strongly

**Table 3. 3.** Eigenvector coefficient of 13 morphological traits for the first three principal components with Eigenvalue, individual and cumulative percentages of the total variance.

| Characteristics | Principal Component | | |
|---|---|---|---|
| | First | Second | Third |
| Growth habit | 0.364 | 0.142 | 0.166 |
| Stem thickness | 0.106 | 0.430 | 0.051 |
| Inter-node length | -0.331 | 0.063 | -0.364 |
| Days to first flowering | 0.339 | 0.111 | 0.389 |
| Days to 50% flowering | 0.328 | 0.124 | 0.390 |
| Branching score | 0.253 | 0.006 | -0.398 |
| Length of leaf rachis | 0.149 | 0.419 | -0.250 |
| Width of leaf rachis | -0.041 | 0.472 | -0.136 |
| Leaflet length | -0.129 | 0.438 | -0.145 |
| Leaflet width | -0.267 | 0.342 | 0.196 |
| Width:length ratio of leaflets | -0.341 | 0.098 | 0.361 |
| Leaflet number per leaf rachis | 0.352 | 0.116 | -0.219 |
| Leaflet shape score | -0.331 | 0.181 | 0.241 |
| Eigenvalue | 6.273 | 4.170 | 1.031 |
| Individual percentage | 48.26 | 32.08 | 7.93 |
| Cumulative percentage | 48.26 | 80.03 | 88.27 |

and positively associated with leaf size (length and width of leaf rachis), leaflet size (length and width) and stem thickness (Table 3.3). Plotting of the accessions across the first 2 PCs (PC1 and PC2), revealed a slight separation of groups across the PC1 axis (Figure 3.1). Accessions with higher values for PC1 (*I. arrecta* 7709, 7524, 9045, 10355, *I. costata* 8712 and *I. crypthantha* 7070) had an erect growth habit, more leaflets per leaf and were late flowering, but had a small leaflet width:length ratio, short internode length, elongated leaflets and narrow leaves. Similarly, accessions with higher values for PC2 (*I. coerulea* 9004, *I. arrecta* 10479 and 10350, *I. amorphoides* 7069, 7549, *I. trita* 9795 and *I. spicata* 13650) were characterised by large leaves and leaflet size (length and width) and thicker stems than accessions with lower PC2 values such as *I. brevicalyx* 7815 and 7848, *I. vicioides* 10486, *I. costata* 8712, *I. arrecta* 7524, *I. spicata* 8254 and 8282).

However, cluster analysis based on morphological characteristics highlighted five main groups and a single outlier (*I. coerulea* 9004) within the *Indigofera* accessions (Figure 3.2). The first level of separation (Group VI *vs* others) was mainly due to stem thickness, leaf width, leaflet size (length and width) and leaflet width:length ratio. The only accession classified in Group VI (*I. coerulea* 9004) had thick stems, large leaves with rounded and large leaflet sizes (length and width) (Table 3.4). The next separation (Group II and V *vs* I, III and IV) occurred on the basis of growth habit and internode length. Groups II and V included all accessions from *I. spicata* and *I. trita*, which had a prostrate growth habit and long internodes as compared to Groups I, III and IV that had either semi-erect or erect growth habit and short or moderate internode length. Group II

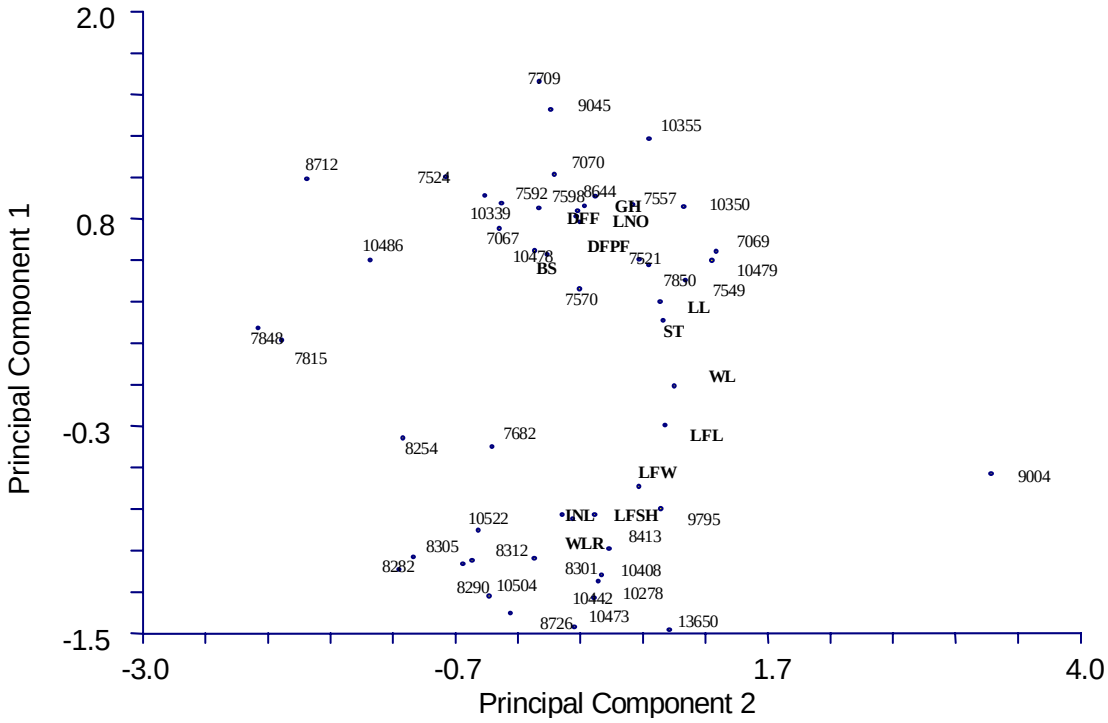



**Figure 3. 1.** Scatter diagram of 41 *Indigofera* accessions and 13 morphological characteristics when plotted against the first two principal components of the correlation matrix (explaining 80.3% of the total variation). GH = growth form; DFF = days to first flowering; DFPF = days to 50% flowering; BS = branching score; ST = stem thickness; LL = length of leaf rachis; LFL = leaflet length; LFW = leaflet width; WLR = width:length ratio of leaflets; INL = inter-node length; WL = width of leaf rachis; LFNO = leaflet number per leaf rachis; LFSH = leaflet shape (see Table 3.2).

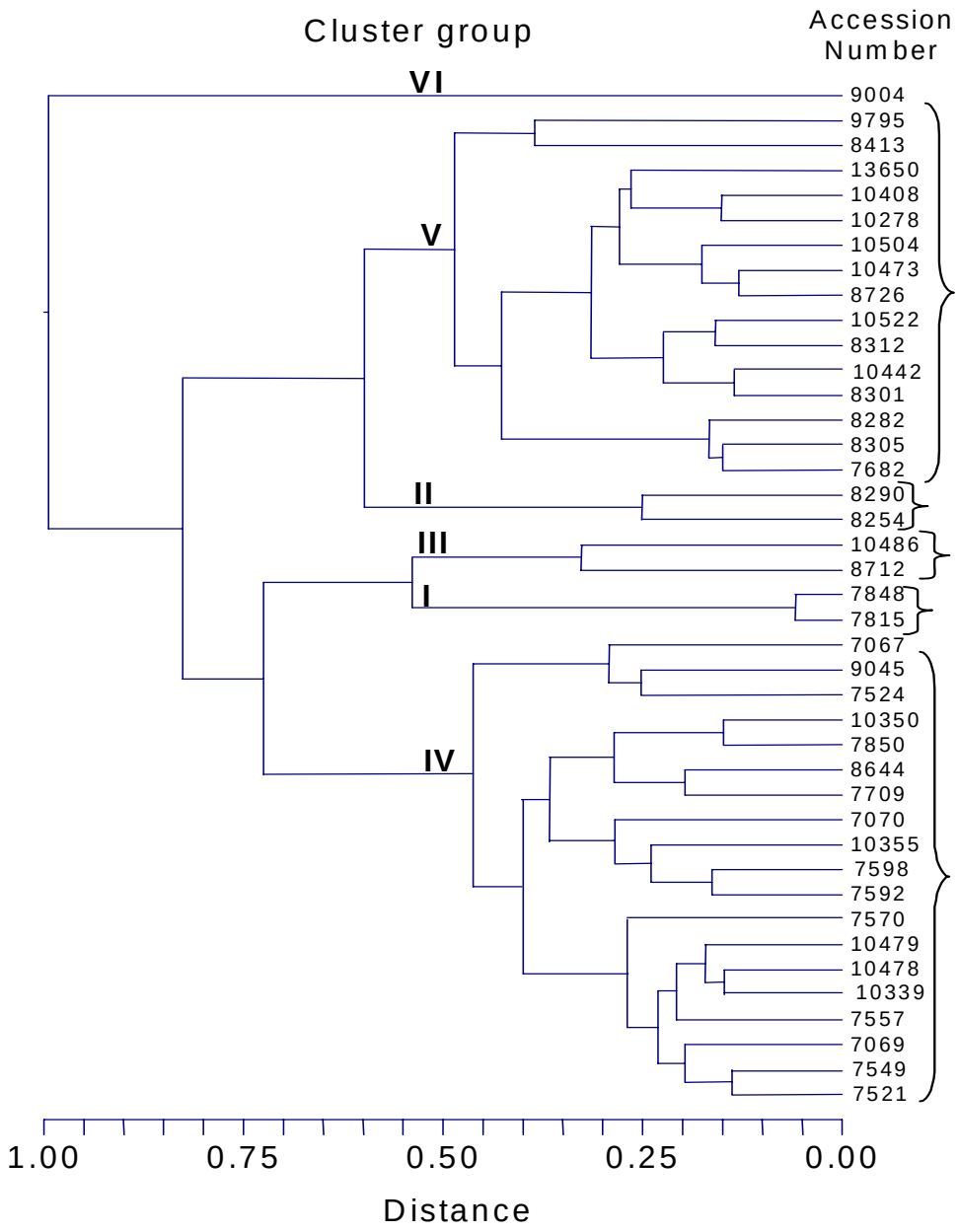



**Figure 3. 2.** Dendrogram of morphological classification of 41 *Indigofera* accessions.

**Table 3. 4.** Variation in morphological characteristics between *Indigofera* accession groups based on clustering of the morphological data set.

| Characteristics | Cluster group | | | | | |
|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI |
| Number of accessions included | 2 | 2 | 2 | 19 | 13 | 1 |
| Number of species included | 1 | 1 | 2 | 3 | 2 | 1 |
| Growth habit | 4.5b[1] | 1.0c | 7.7a | 7.9a | 1.6c | 8a |
| Days to first flowering[2] | 91b | 94.5b | 148.8a | 131.1a | 89.4b | 145a |
| Days to 50% flowering | 103.7b | 120.3b | 153a | 142.6a | 107.7b | 160a |
| Branching | 5.1bc | 8.3a | 5.8abc | 6.7ab | 4.3c | 3.5c |
| Length of leaf rachis | 22.6bc | 30.8bc | 15.9c | 58.4a | 38.2b | 73.4a |
| Width of leaf rachis | 15.1d | 22.4cd | 12.5d | 29.7b | 28.2bc | 50.6a |
| Leaflet number per leaf rachis[2] | 11.1b | 7.8c | 7.8c | 15.7a | 7c | 8bc |
| Leaflet length[2] | 7.6d | 11.5cd | 7.3d | 14.7bc | 15.9b | 23.8a |
| Leaflet width | 2.5d | 5.9c | 3.2d | 5.4c | 8.6b | 17.6a |
| Width: length ratio of leaflets[2] | 0.355d | 0.510bc | 0.435c | 0.365d | 0.551b | 0.730a |
| Leaflet shape | 3c | 4.3b | 3.8b | 3.9b | 6a | 7a |
| Stem thickness[2] | 0.63d | 1.65b | 1.35c | 1.86b | 1.40c | 3.25a |
| Inter-node length[2] | 10.2b | 22.3a | 2.6c | 9.9b | 22.4a | 8.9bc |

[1]Within a row, means followed by different letters differ significantly at $P<0.05$.

[2]Mophological characteristics used for cluster analysis of morphological data set.

included the two accessions of *I. spicata* (8290 and 8254) known for their big stem diameter, excellent branching, slightly elongated leaflets and smaller leaflet size (length and width) than accessions of *I. spicata* in Group V (Table 3.4), which are less branched, with slightly rounded and large leaflet size and stem diameter. Group IV included accessions from *I. arrecta*, *I. cryptantha* and *I. amorphoides*, which differed from accessions in Groups III and I, mainly due to their bigger leaves and leaflets (length and width), more leaflets per leaf with big stem diameter. The last separation, between Groups III and I, was mainly due to growth habit, days to first and 50% flowering, leaflet number, leaflet width:length ratio, leaflet shape, stem diameter and internode length (Table 3.4). The two accessions in Group III (*I. vicioides* 10486 and *I. costata* 8712) had relatively erect growth habits, late flowering period, few leaflets per leaf that are slightly elliptical in shape, thick stems and shorter internodes than accessions in Group I (*I. brevicalyx* 7815 and 7848) characterised by semi-erect growth form, early flowering habit, a relatively elongated leaflets, thin stem diameter and longer internodes.

### *3.4.2.* ***Analysis of agronomic characters***

Principal component analysis of the agronomic data set revealed only one PC with Eigenvalues greater than 1. The first PC (PC1) accounted for 82.9% of the total variation, and the second PC (PC2) explained only 9.6% of the total variation. Thus, the first 2 components accounted for 92.5% of total variance. The remaining six components contributed only 7.5% (Table not presented).

All characteristics contributed equally to the first PC, but some were in different directions. No single characteristic appeared to be a dominant trait that could explain most of the variation across PC1. The variables separating accessions across the PC1 axis with corresponding Eigenvectors in parenthesis were: leaf yield (-0.338), stem yield (-0.363), total dry matter yield (-0.363), leaf percentage (0.330), plant height or length of the principal stem (-0.362), canopy diameter at widest point (-0.345), canopy diameter at right angle to the widest point (-0.366) and mean canopy diameter (-0.358). Traits that separate accessions along the PC2 axis (Eigenvector in parenthesis) were: leaf yield (-0.405), stem yield (-0.329), total dry matter yield (-0.369), leaf percentage (0.084), plant height or length of the principal stem (-0.121), canopy diameter at widest point (0.504), canopy diameter at right angle to the widest point (0.358) and mean canopy diameter (0.436). The second PC was dominated by canopy diameter and yield characteristics, these two traits contributing in different directions. Thus, accessions with similar response in terms of characteristics that dominated PC1 and PC2 are grouped in close proximity in the 2-dimensional space (Figure 3.3). Those accessions with higher values for PC1 displayed relatively poor dry matter yields, short plant heights and small canopy diameter, but their dry matter was leafier. Accessions with higher values of PC2 (*I. trita* 9795, *I. spicata* 10442, 8290, 8254, 8312, 8413, *I. arrecta* 10339, 10478 and *I. amorphoides* 7557) were poor in terms of biomass production but they have larger canopy diameters than accessions with lower PC2 values (*I. vicioides* 10486, *I. arrecta* 7709, 9045, 10350, *I. coerulea* 9004, *I. cryptantha* 7067 and 7069) that display, in contrast, high biomass yield from smaller canopy spread diameter.

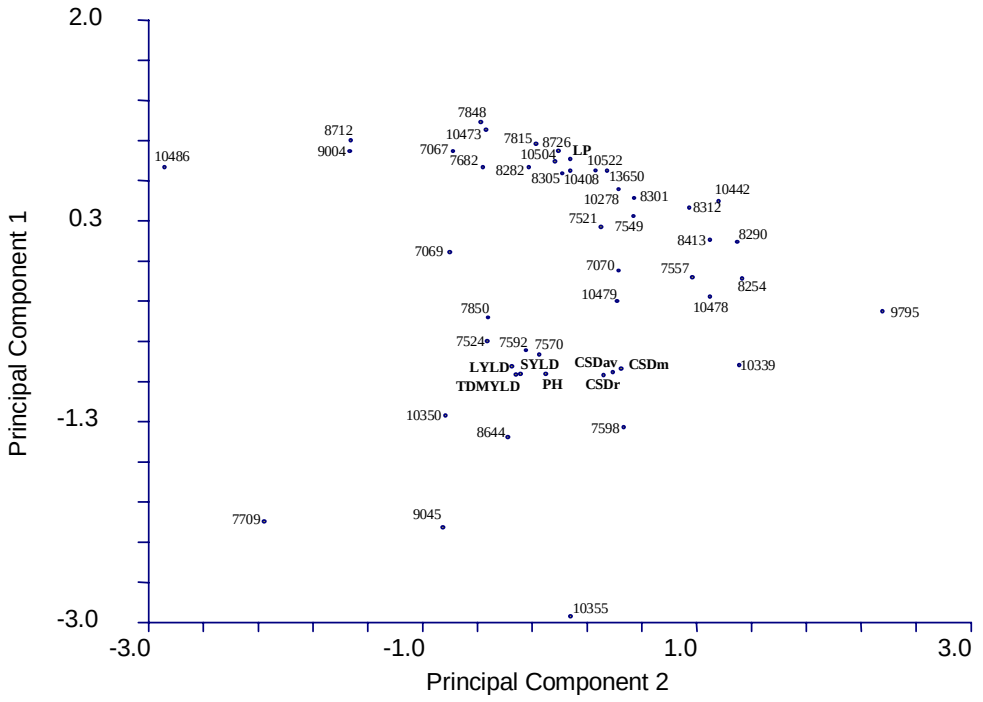



**Figure 3. 3.** Scatter diagram of 41 *Indigofera* accessions and eight agronomic characters when plotted against the first two principal components of the correlation matrix (explaining 92.5% of the total variation). LP = leaf percentage; LYLD = leaf DM yield; SYLD = stem DM yield; TDMYLD = total DM yield; PH = plant height/stem length; CSDm = maximum spread diameter; CSDr = spread diameter (right angle to the maximum); CSDav = mean spread diameter (see Table 3.2).

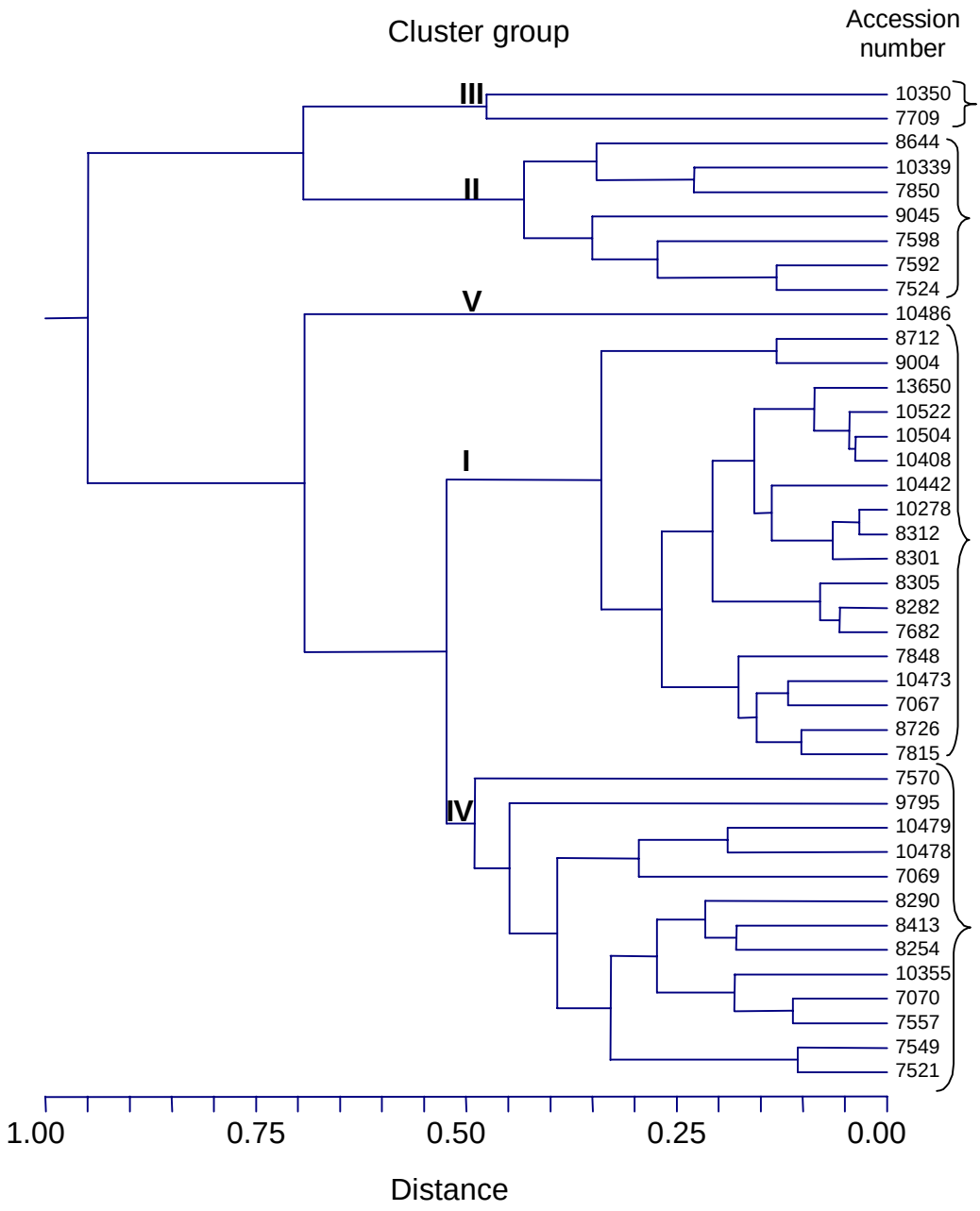



**Figure 3. 4.** Dendrogram of agronomic classification of 41 *Indigofera* accessions.

Clustering, using unweighted average linkage algorithms, however, revealed five main agronomic groups (Figure 3.4). The first separation was due mainly to plant height, percentage leaf and canopy spread. Thus, accessions in Groups II and III were composed of taller plants with a lower percentage of leaf and greater canopy diameter than accessions in Groups I, IV and V (Table 3.5). Group III included the two tallest, and perhaps high leaf-yielding, accessions of *I. arrecta* (7709 and 10350) with large canopy diameter. The next separation, between Group V and the remaining groups (Groups I and IV), appeared to be due to leaf dry matter yield. The only accession in Group V (*I. vicioides* 10486) had a relatively high leaf yield compared with accessions in Groups I and IV. Most accessions from morphological Group V and some from Groups I, III, IV and VI are included in agronomic Group I, which is characterised by low stem yield, low total dry matter yields, short plants and smaller canopy diameters as compared to accessions in Group IV that are relatively taller, high in stem and total dry matter yield with large canopy diameter.

### *3.4.3.* ***Combined analysis of morphological and agronomic characters***

In order to identify a core set of attributes, for use in future screening evaluations, and to determine the effective contribution of different characteristics to variation, characteristics with higher coefficients for each component with Eigenvalue below 0.70 were discarded from the morphological and agronomic data sets as proposed by Jolliffe (1972; 1973), and applied by Veasey *et al*. (2001).

**Table 3. 5.** Variation in agronomic characteristics between *Indigofera* accession groups based on clustering of the agronomic data set.

| Characteristics | Cluster group | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | V |
| Number of accessions included | 18 | 7 | 2 | 13 | 1 |
| Number of species included | 5 | 1 | 1 | 5 | 1 |
| Plant height [2] | 25.2d[1] | 82.3b | 147.6a | 55.3c | 17.5d |
| Total DM yield | 20.9c | 357.9a | 645.1a | 133.2b | 220ab |
| Stem DM yield | 7.2d | 209ab | 413.6a | 48.1c | 50.1bc |
| Leaf DM Yield | 13.7c | 148.9b | 231.5a | 85.2c | 169.9ab |
| Leaf percentage[2] | 68.0a | 42b | 35.9b | 64.9a | 77.2a |
| Canopy diameter (at the widest point) | 42d | 83.4b | 105.1a | 70.2c | 18d |
| Canopy diameter (at right angle to the widest) | 32.4d | 77.1b | 96.2a | 59.9c | 17d |
| Mean canopy diameter[2] | 37.2d | 80.3b | 100.6a | 65.1c | 17.5d |

[1]Within a row, means followed by different letters differ significantly at $P<0.05$.

[2]Agronomic characteristics used for cluster analysis of agronomic data set.

Accordingly, nine morphological characteristics were eliminated in the following order: internode length, leaflet shape, stem thickness, leaflet number, leaf length, days to first flowering, leaflet width, leaf width and leaflet width:length ratio. The characteristics selected (growth habit, days to 50% flowering, branching score and leaflet length) were highly correlated ($P<0.001$) with at least one of the discarded morphological characteristics. Likewise, for the agronomic data set, six characteristics (including percentage leaf and plant height) were identified initially, but four characteristics were discarded in the following order: stem yield, canopy diameter at right angle to the widest point, mean canopy diameter and total dry matter yield. The two characteristics were retained to keep the recommended minimum number of characters necessary for cluster analysis (Mardia *et al.* 1979, cited by Veasey *et al*. 2001; Strapasson 1997, cited by Veasey *et al*. 2001). The selected agronomic characteristics were leaf yield, plant height or length of the principal stem, percentage leaf and canopy diameter at the widest point. The selected characteristics were highly correlated ($P<0.001$) with the discarded agronomic characteristics.

The selected eight characteristics (four morphological and four agronomic) were combined to create a new principal component and cluster analysis. The combined (morphological and agronomic) principal component analysis showed that the first-two components with Eignvalues greater than 1 accounted for 73.9% of the total variation (Table 3.6). The first component, which explains 59% of the total variation, was positively associated with leafiness and negatively with leaf yield, plant height, days to 50% flowering, growth habit, canopy diameter at the widest point and branching score.

**Table 3. 6.** Eigenvector coefficient of eight selected descriptor traits for the first-three principal components with Eigenvalue, individual and cumulative percentage of the total variance.

| Characteristics | Principal Component | | |
|---|---|---|---|
| | First | Second | Third |
| Growth habit | -0.357 | -0.215 | -0.515 |
| Days to 50% flowering | -0.388 | -0.196 | -0.405 |
| Branching score | -0.322 | -0.192 | 0.494 |
| Leaflet length | 0.018 | 0.828 | -0.338 |
| Leaf yield | -0.421 | -0.073 | -0.076 |
| Leaf percentage | 0.371 | -0.126 | -0.107 |
| Plant height/Length of principal stem | -0.421 | 0.199 | 0.026 |
| Canopy diameter (at right angle to the widest) | -0.356 | 0.363 | 0.442 |
| Eigenvalue | 4.719 | 1.196 | 0.907 |
| Individual percentage | 58.98 | 14.95 | 11.33 |
| Cumulative percentage | 58.98 | 73.93 | 85.26 |

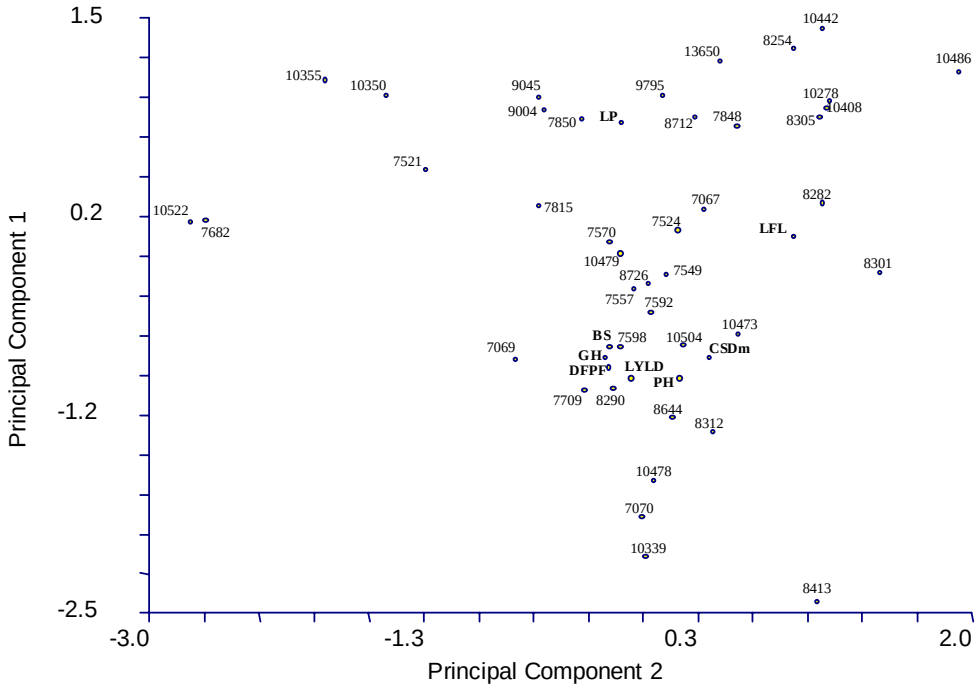



**Figure 3. 5.** Scatter diagram of 41 *Indigofera* accessions and eight selected morphological and agronomic attributes when plotted against the first-two principal components of the correlation matrix (explaining 73.9% of the total variation). LP = leaf percentage; LFL = leaflet length; LYLD = leaf DM yield; BS = branching score; GH = growth form; PH = plant height/stem length; DFPF = days to 50% flowering; CSDm = maximum spread diameter (see Table 3.2).

PC2 was mainly dominated by leaflet length and canopy diameter at the widest point. Accessions with a similar response in terms of characteristics that dominated PC1 and PC2 are grouped in close proximity in the 2-dimensional space (Figure 3.5). Those accessions with higher values for PC1 (*I. spicata* 10442, 8254, 13650, 10278, 10408, 8305, *I. vicioides* 10486, *I. arrecta* 9045, 10350, 10355, 7850, *I. costata* 8712, *I. brevicalyx* 7848, *I. coerulea* 9004, and *I. trita* 9795) were leafier (Figure 3.5), but had lower leaf yields, were shorter, had early flowering habit, had a semi-erect to prostrate growth habit and small canopy diameters and were poorly branched. Similarly those accessions with lower values of PC1 (*I. spicata* 8413, 8290, 8312, *I. arrecta* 10339, 10478, 7709, 8644 and *I. amorphoides* 7070) had high leaf yields, taller, late flowering, erect with large canopy spread diameter and good branching but the dry matter is more stemmy.

Clustering of the selected morpho-agronomic characteristics based on unweighted average linkage algorithm highlighted eight main groups of *Indigofera* accessions (Figure 3.6). The first level of separation was based on biomass yield and leafiness of the biomass. Accessions classified in Groups V and IV were tall, had higher stem and total dry matter yields and were less leafy than accessions in the remaining groups (Table 3.7). The three *I. arrecta* accessions (10350, 8644 and 7709) classified in Group V differed from the other six *I. arrecta* accessions (7850, 10339, 7598, 7592, 9045 and 7524) classified in Group IV by being later-flowering, taller plants, with larger canopy spread and lower leaf percentage. Among the remaining groups, the only accession classified in Group VIII (*I. coerulea* 9004) differed from accessions in Groups I, II, III, VI and VII on

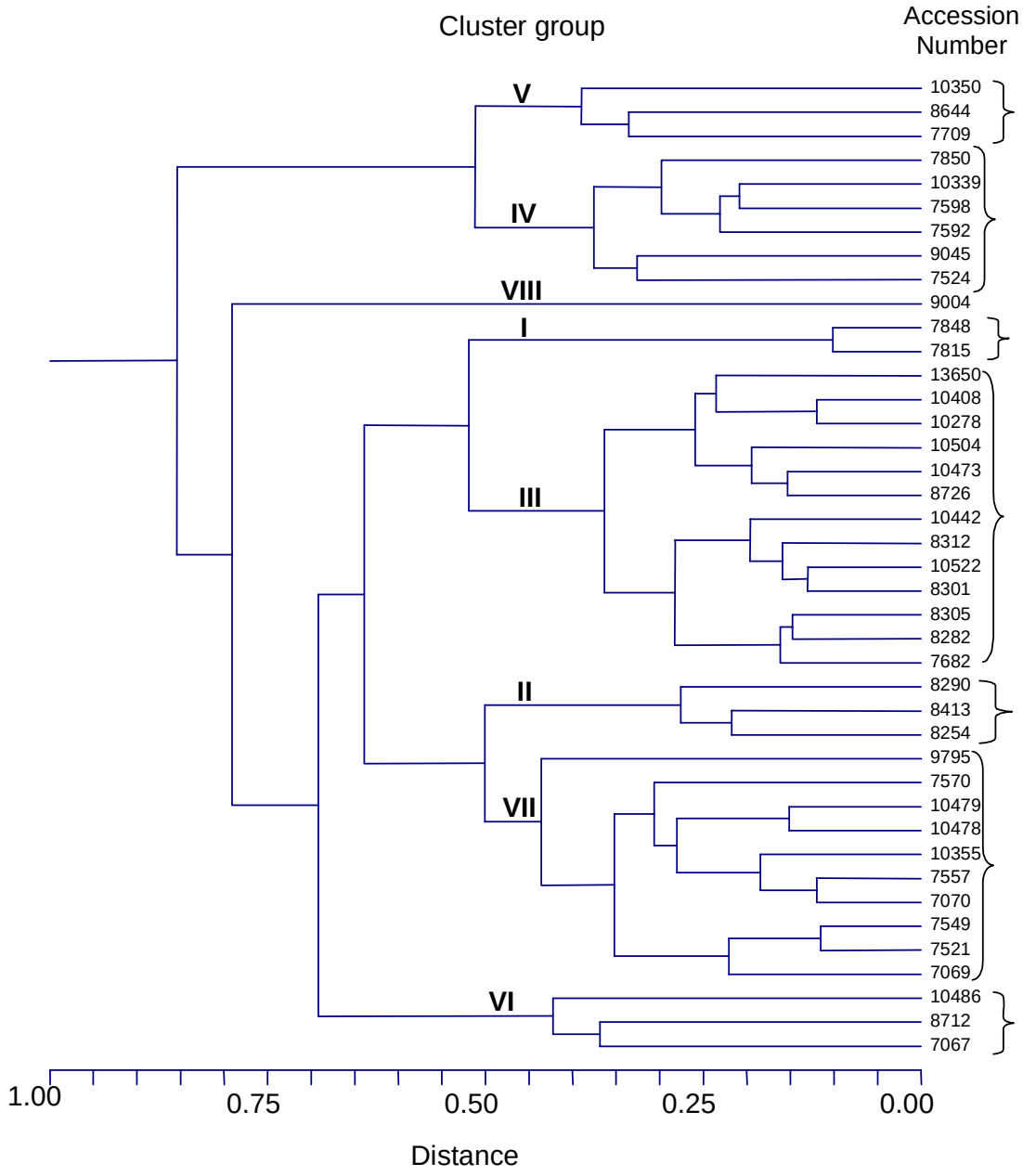



**Figure 3. 6.** Dendrogram of morpho-agronomic classification of 41 *Indigofera* accessions.

**Table 3. 7.** Variation in morphological and agronomic characteristics between *Indigofera* accession groups based on clustering of selected morphological and agronomic descriptors.

| Character | Cluster group | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII | VIII |
| Number of accessions | 2 | 3 | 13 | 6 | 3 | 3 | 10 | 1 |
| Number of species included | 1 | 1 | 1 | 1 | 1 | 3 | 4 | 1 |
| Days to first flowering | 91d[1] | 95.2d | 86.8d | 134.6b | 153.7a | 143.2ab | 120.6c | 145ab |
| *Days to 50% flowering*[2] | 103.7d | 121.1c | 105.5d | 149.3b | 165.3a | 149b | 129.8c | 160ab |
| *Plant height* [2] | 19.9ef | 38.9d | 26.8e | 78.5b | 133.6a | 18.9f | 60.2c | 26ef |
| Total DM yield | 12.1e | 66.5d | 15e | 317.6a | 629.9a | 108cd | 153.2bc | 53.6d |
| Stem DM yield | 2.6c | 25b | 5.2c | 176.4a | 410.7a | 28.2b | 55b | 22.9b |
| *Leaf DM yield*[2] | 9.5ef | 41.5d | 9.8f | 141.2ab | 219.2a | 79.8cd | 98.3bc | 30.8de |
| *Leaf percentage*[2] | 78.9a | 61.7bc | 67.3bc | 43.6d | 34.8e | 70.5ab | 65.9bc | 57.4c |
| *Growth habit*[2] | 4.5c | 1.2d | 1.3d | 8.1ab | 9a | 7.8b | 7.2b | 8ab |
| *Spread diameter (widest point)* [2] | 33.4cd | 71.7b | 47.4c | 81b | 102.8a | 23.4d | 69.8b | 21.5d |
| Spread diameter (right angle to the widest) | 28.2de | 59.8c | 35.5d | 74.4b | 95.1a | 21.2e | 60c | 19e |
| Mean spread diameter | 30.8de | 65.7bc | 41.4d | 77.7b | 99a | 22.3e | 64.9c | 20.3e |
| *Branching*[2] | 5.1bc | 8.1a | 3.9c | 6.7ab | 6.9ab | 5.2b | 6.8ab | 3.5c |
| Leaf length | 22.6c | 34.1c | 36.3c | 49.1b | 57.6ab | 25.4c | 65.7a | 73.4a |
| Leaf width | 15.1d | 24.9c | 27.6c | 27.2c | 28.2bc | 16.4d | 32.6b | 50.6a |
| Leaflet number per leaf rachis | 11.1b | 7.6bc | 7c | 14.9a | 17.6a | 11b | 14.7a | 8bc |
| *Leaflet length*[2] | 7.6d | 13c | 15.7bc | 13.6c | 13.9bc | 8.7d | 16.3b | 23.8a |
| Leaflet width | 2.5e | 7.1bc | 8.5b | 4.9d | 4.5d | 3.6de | 6.4c | 17.6a |
| Width: length ratio of leaflets | 0.335cd | 0.547b | 0.550b | 0.360cd | 0.327d | 0.420c | 0.391c | 0.730a |
| Leaflet shape | 3e | 5.1bc | 5.9ab | 3.7de | 3.5de | 4cde | 4.4cd | 7a |
| Stem thickness | 0.63e | 1.75bc | 1.34d | 1.68bc | 1.82b | 1.4cd | 1.99b | 3.25a |
| Inter-node length | 10.2bc | 23.2a | 21.5a | 9.83bc | 7.31c | 3.71c | 13.3b | 8.85b |

[1]Within a row, means followed by different letters differ significantly at P<0.05.

[2] Characteristics selected as descriptors for the analysis of combined data set (Italicised).

the basis of stem thickness, wider leaf width and leaflet size (length and width) and high leaflet width:length ratio. Group VI, consisting of accessions from three different species (*I. vicioides* 10486, *I. cryptantha* 7067 and *I. costata* 8712), was differentiated from the remaining groups (I, II, III and VII) by their late flowering characteristics. Among the remaining groups, Groups III and I were relatively short, had low leaf, stem and total dry matter yields, small canopy spread diameter, thinner stems and early flowering than accessions in Groups II and VII. The two accessions of *I. brevicalyx* (7848 and 7815), belonging to Group I, had a semi-erect growth habit, were leafier with relatively small size and elliptic leaflets, had few leaflets per leaf with narrow leaf width, thinner stem with smaller internodes than accessions in Group III that have an almost prostrate growth habit, large leaflet size (length and width), longer internodes and thick stems. Group III included 13 accessions from *I. spicata*, while the remaining accessions of *I. spicata* were included in Group II. Accessions of *I. spicata* in Group III differ from accessions of *I. spicata* in Group II, mainly due to shorter days to maturity (50% flowering), short principal stem length, low leaf, stem and total dry matter yield, small canopy spread diameter, fewer branching and small stem diameter. Group VII included 10 accessions from four different species (*I. amorphoides*, *I. crypthantha*, *I. arrecta* and *I. trita*) and were dissimilar from accessions in Group II mainly due to their erect growth habit, longer days to first flower appearance, higher leaf and total dry matter yield, large leaf size (length and width), more leaflets per leaf, greater leaflet length, smaller leaflet width:length ratio and shorter internodes.

### 3.5. Discussion

Knowledge of the existing genetic variation between various morphological and agronomic traits is vital for any plant improvement and breeding program. Information that will be obtained through multivariate techniques such as PCA and cluster analysis may assist plant breeders in the characterisation of germplasm to explore the presence of genetic variation (van de Wouw 1999), to identify valuable characteristics, which account for genetic variation (Veasey *et al.* 2001; Nunes and Smith 2003) and to find a limited number of highly differentiated populations for use in programmes of crossing and selection (Veronesi and Falcinelli 1988).

In this study, the variance accounted for by the first-two components for the morphological and agronomic data sets was relatively high (>80%). According to Veasey *et al.* (2001), this explains satisfactorily the variability manifested between individuals. Morphological traits such as growth habit, number of leaflets per leaf, days to flowering, branching, and leaflet shape and leaf size had shown a strong contribution to PC1 and PC2 axis. Thus, improvement of these species is possible by selecting valuable morphological characteristics with agronomic significance. Productive species for high potential areas of Ethiopia, with high rainfall and long growing seasons, can be identified through selection of individuals with higher values of PC1 and PC2 in the morphological data sets. By contrast, in spite of lower productivity, individuals with lower PC1 and PC2 values could best be used, because of their prostrate growth habit and early flowering nature, as early season pasture legumes or in areas where overgrazing is the major constraint to the establishment of erect species. Likewise, PC analysis of the agronomic

data set has demonstrated the variability manifested between individuals for important agronomic traits linked to forage and cover crops. Although PC2 explained only 9.6 % of the total variation in the agronomic data sets, the fact that biomass yields and canopy diameter contributed in a different direction to PC2 suggests the possibility of developing forage varieties by selecting individuals or accessions with lower PC2 (high in biomass production and small canopy size).

Clustering techniques were employed to estimate genetic distance and classify accessions into relatively homogenous groups. In this study, cluster analysis revealed fairly distinct species patterns. On the basis of morphological characters alone, grouping of the accessions revealed more inter-species variation with the exception of two accessions of *I. spicata* (8254 and 8290) classified in a different group from the other 11 accessions of *I. spicata*, due mainly to excellent branching characteristics, relatively late flowering habit and small leaflet with slightly elongated leaf shape. Grouping of accessions on the basis of agronomic characters, however, revealed both inter- and intra-species variability. *I. vicioides* (10486) was classified into a different group, among leafier accessions, due to its superior leaf dry matter yield. These characteristics make the species a suitable forage plant that needs to be incorporated in future plant introduction and evaluation studies. Within a species, accessions from *I. spicata* and *I. arrecta* were categorised into different agronomic main groups suggesting that there is genetic variation that could be exploited through direct selection of accessions for higher fodder yield. Variation in dry matter yield was also reported among *Astragalus hamosus* populations, which was mainly related to climatic conditions at original sites (Zoghlami and Zouaghi 2003). In this

study, clustering on the basis of morphological traits alone failed to consistently and satisfactorily reveal variation between accessions in terms of agronomic performance. A significant number of accessions from the same morphological main groups were categorised into a different agronomic main group. Hence, agronomic characters should complement the classification of the accessions in order to reveal variation between accessions that will have importance in relation to future germplasm utilisation for forage breeding endeavours.

Grouping of accessions based on morpho-agronomic descriptors revealed both inter- and intra-species variability for all studied morphological and agronomic traits (Table 3.7). Once again, the two *I. spicata* accessions (8254 and 8290) were categorised together with 8413 in a different morpho-agronomic group from the other 10 accessions of *I. spicata* (Group III) suggesting that there is genetic variation in terms of some morphological and agronomic traits. Two of the 3 *I. spicata* accessions in group II (8254 and 8413) originated from a highland area high with rainfall (Table 1). Early flowering characteristics of the latter (Group III) make them potentially valuable materials for developing varieties that can be used as cover crops for quick soil stabilisation for erosion control or rehabilitation of over-grazed areas and terrace banks. Similarly accessions in morpho-agronomic Group II are valuable material for developing *I. spicata* varieties for better rainfall areas due to late flowering habit, excellent branching, large canopy diameter, high leaf, stem and total dry matter yield. Lane *et al.* (2000) indicated that leaf size was a commonly used character for categorising white clover populations, because it was strongly correlated with important morphological characters such as stolon density

and branching (Jahufer *et al*. 1994), plus root type and diameter (Caradus 1990), which are likely to affect leaf biomass production. Accessions of *I. arrecta* showed considerable variability for most of the morpho-agronomic traits studied. The broad range in group means of morpho-agronomic groups IV, V and VII for some traits, related to high leaf dry matter yield and interception of rainfall, confirms the possibility to develop high yielding varieties of *I. arrecta* that will have roles both as forage and cover crops. Most of the high yielding accessions originated either from a highland area or from a sub-humid mid-altitude area reciving high rainfall. The two accessions of *I. cryptantha* were from different populations as they were classified in different morpho-agronomic groups (VI *vs*. VII), due mainly to variation in days to both first flowering and 50% flowering. Flowering date is an important, well recognised adaptive characteristic and variation in the time of flowering has been reported in *Trifolium glomeratum* (Smith *et al*. 1995), *T. subterraneum* (Cocks and Philippes 1979) and *Medicago polymorpha* (Brock *et al.* 1971). Although the composition of the groups could not be related closely to the limited information available on their geographical or climatic origin, other studies indicated that genetic variation for flowering time is related to the origin of accessions. According to Loi *et al.* (1999) and Bennett (1997), a harsh environment exerts strong selection pressures on populations, favouring individuals which flower and set seed within a short growing season. Almost all of the *I. amorphoides* accessions originated from the lowland areas. Less genetic variability was observed between accessions of *I. amorphoides*, which were included in the same newly formed morpho-agronomic groups (Group VII) and also previously in the same morphological (Group IV) and agronomic main groups (group IV).

The characterisation of this group of *Indigofera* accessions has improved the knowledge of the species, thereby facilitating the identification of materials with desirable characteristics, *e.g*. high leaf yield for fodder production and large canopy diameter for interception of rainfall as a cover crop as well as useful morphological characteristics with agronomic significance. Morphological and agronomic characteristics, which underlie major variability, have also been identified. The selected characteristics could explain variability between accessions of *Indigofera* germplasm and can be used as a core set of descriptor traits in future evaluation studies and breeding programs.

This study also confirmed that accessions of *Indigofera* species display a large degree of variation for studied morpho-agronomic traits. The broad trait diversity evident among the accessions of *I. spicata*, *I. arrecta* and *I. cryptantha* suggests ample opportunity for genetic improvement of those plant species through selection directly from the accessions. Grouping accessions into morphologically similar, and possibly genetically similar groups (Souza and Sorrells 1991) is helpful for germplasm collection and selecting parents for crossing. In addition, the study allowed the selection of promising accessions from the different agro-ecological sites in Ethiopia, which will be studied further for their likely toxicity due to indospicine accumulation. Subsequently, the grouping of accessions by phenotypic diversity in the present study and the data from the indospicine study will be used to classify the accessions into distinct morphological and toxicity levels, which could be used for various breeding, collection and conservation programs.

# CHAPTER 4

# Forage production and potential nutritive value evaluation of 24 shrub type *Indigofera* accessions grown under field conditions

## 4.1. Abstract

Twenty-four shrubby *Indigofera* accessions from seven species were evaluated in terms of their forage production, potential nutritive value and toxicity of the forage biomass. Eighteen seedlings were transplanted in field plots measuring 1.5 x 3m in January 2003. Spacings of 50 cm between and within rows were maintained. Each accession was planted in three replicates. In both the establishment and second season differences between and within species for plant height, canopy spread diameter, fodder yield and leaf percentage of the biomass were significant ($P < 0.05$). *I. amorphoides* 7570, *I. cryptantha* 7070 and *I. arrecta* 7709 were superior in terms of forage yield in the first season, while *I. amorphoides* 7549, *I. cryptantha* 7067 and *I. arrecta* 10350 were superior in the subsequent season. Higher crude protein (CP) concentrations (g $kg^{-1}$ DM) were recorded for *I. cryptantha* (298.7) and *I. amorphoides* (276.8), while the lowest were recorded for *I. coerulea* (159.2) and *I. vicioides* (200.6). Phosphorous (P) concentrations (g $kg^{-1}$ DM) of the forage biomass were higher in *I. cryptantha* (3.72), *I. brevicalyx* (3.50) and *I. amorphoides* (3.26) than in *I. costata* (2.30). The in *vitro* organic matter digestibility (IVOMD) (g $kg^{-1}$ DM) was higher in *I. amorphoides* (748.3) and *I. cryptantha* (736.4) than in *I. brevicalyx* (638.2) and *I. costata* (654.6). Remarkable differences were observed, both between and within a species, in terms of indospicine level of the forage biomass. Among the species, the level of indospicine in *I. brevicalyx* was insignificant (0 to 2 mg $kg^{-1}$ DM), while it was minimal in *I. coerulea* (23.0) and *I. cryptantha* (35.4), and moderate in *I. arrecta* (126.1), *I. costata* (135.9) and *I. amorphoides* (180.8) compared to the levels in *I. vicioides* (705.6). Variability within a species for nutritive value parameters, and level of indospicine, were significant suggesting the possibility of directly selecting accessions with forage potential for subsequent evaluation with target animals.

**Key words:** Accessions, forage, *Indigofera*, indospicine, nutritive value, shrub-type.

## 4.2. Introduction

Indigenous fodder trees and shrubs are well known for their benefit as sources of feed during the driest months and drought periods across the semi-arid and arid areas of the tropics and sub-tropics. Many of these are, however, problematic as a feed supplement as they often contain anti-nutritional compounds, which are, either toxic to rumen microbes or to the animal, or their metabolic products are toxic (D'Mello 1992; Lowry *et al.* 1996).

Amongst the native flora *Indigofera* species are known for their excellent adaptability in a range of environments (Hassen *et al.* 2005 Unpublished data), with diverse morphological and agronomic attributes significant to their use as forage and cover crops (Hassen *et al*. 2005 Unpublished data). The shrubby types are generally superior to prostrate type in terms of their biomass production and remarkable variation existed between and within species (Hassen *et al*. 2005 Unpublished data). However, little is known about their variation in terms of winter survival, forage production in subsequent seasons, potential nutritive value and anti-nutritional compounds, which may limit the feeding value of the forage. Previous studies had indicated that a species, such as *Indigofera spicata*, contains the free amino acid called indospicine, which causes hepato-toxicity when grazed by cattle (Norfeldt *et al*. 1952) or fed to chicks (Britten *et al.* 1963), rabbits (Hutton *et al*.1958a), mice (Hutton *et al.* 1958b) or rats (Christie *et al*. 1975). However, among collections evaluated in Australia, genetic variation between and within species was significant (Williams 1981; Strickland *et al*. 1987), suggesting the need for screening more materials before promoting the species widely as forage crops.

Chemical analyses, particularly in combination with *in vitro* digestibility and the determination of the indospicine level in the leaf biomass, can help to assess the potential nutritive value of species/accessions at a preliminary stage of evaluation for use as forage plants. The present study evaluated 24 shrub type accessions of *Indigofera*, from seven species, with an aim to study variation in forage biomass production and winter survival at Pretoria, and to assess potential nutritive value of the leaf biomass as a forage source for both livestock and game.

## 4.3. Material and Methods

### *4.3.1* ***Location, field lay out and management***

The field experiment was carried out on the Hatfield Experimental Farm, University of Pretoria (1370 m asl). Seeds of 24 shruby *Indigofera* accessions were sown in trays in a nursery. After establishment, 54 seedlings of each accession were transplanted into field plots in January 2003. Eighteen seedlings were planted in 1.5 m x 3 m plots with a spacing of 50 cm between rows and plants. Each accession was replicated 3 times. Spacings of 50 and 100 cm were maintained between adjacent plots and blocks, respectively. The plants were irrigated twice per week for 2 hours depending on rainfall events. Plots were kept weed-free by hand pulling.

A total of five middle plants, including one border plant, per plot per replication were considered for estimation of dry matter yield with a harvestable plot area of 1.25 $m^2$. In the first growing (2002/2003) season all plants were harvested at the 50% flowering stage to a height of 15cm. Subsequently all plots were clear cut to the same height before the

commencement of winter (June 2003), and left to grow to determine winter survival and biomass production in the subsequent season. In the 2003/2004 growing season, however, all accessions were harvested at the same time, between 15 and 18 March 2004. The harvested materials were separated into leaf and stem components, which were dried in a forced-draught oven at 70 °C for 48 hours to determine moisture content of the biomass.

### *4.3.2.* ***Chemical composition and*** *in vitro* ***digestibility***

Samples of leaf biomass harvested in year 2003 were milled to pass through a 1 mm sieve and representative sub-samples were stored in airtight containers for subsequent laboratory analyses. The DM, ash and Nitrogen concentrations of the sample were determined following standard procedure (AOAC 2000). Crude protein was determined from N concentration by multiplying with a factor of 6.25. Phosphorus content was determined following AOAC (2000) procedures. *In vitro* organic matter digestibility (IVDOM) was determined by the Tilley and Terry (1965) procedure, as modified by Engels and Van der Merwe (1967).

### *4.3.3.* ***Indospicine determination***

Indospicine analyses were done on dried and milled leaf material in triplicate. The analysis involved three stages: plant extraction, solid phase extraction and ninhydrine test. It began with weighing 0.5 g of dried sample and mixing the sample with 5ml of Methanol: Chloroform: 2% Ammonia (12:5:3) (MCA) solution in a test tube. The cells were ruptured by Branson sonifier model B-30 (20% duty cycle, level 2 output) with microtip for one minute. The supernatant was then collected following centrifugation at

3000 rpm for four minutes. The remaining pellet was re-extracted twice with 3ml of MCA solution. The supernatants were pooled and 1.5ml of chloroform and 2.25ml of water was added to the mixture and centrifuged to separate the upper phase from the lower. The upper phase (about 12 ml) was collected and the volume reduced with a Buchi apparatus to 8 ml. The remaining solvents were subsequently removed by placing in an oven set at 105 °C until the volume was reduced to about 2ml.

The second stage was solid phase extraction of the sample. The strong base property of indospicine was exploited to bind it to a weak cation exchanger under high pH and low ionic strength conditions. It was eluted under high ionic strength conditions. It was confirmed that these conditions effectively bind and elute arginine, the structural analogue of indospicine. For this a new Isolute CBA (2g/15ml) column (Biotag, Uppsala, Sweden) was rinsed with 5ml of methanol to wet the column, and subsequently by 30ml of 0.1M carbonate buffer (pH 10) to change the pH of the column to 10 and finally rinsed with 30ml of 0.01M carbonate buffer at pH 10. The sample was reconstituted to 15ml with 0.01M carbonate buffer solution, the pH adjusted to pH 10 by adding a quantified volume of 1M sodium hydroxide and filtered to prevent blocking of the column by insoluble particles. The sample was then loaded into the column, subsequently rinsed with 5ml of 0.01M carbonate buffer to remove unbound components. The indospicine was eluted thereafter with 15ml of 0.1M carbonate buffer and the eluent was collected and subsequently dried in an oven at 105 °C. The column was rinsed again, using 10ml of 0.1M carbonate buffer for reuse.

The third stage involved a ninhydrin test. For this a 2 % ninhydrine solution was prepared using acetone. The dried sample was reconstituted with 1 ml of acetate buffer and the pH was adjusted to 4.5 by adding a known amount of acetic acid. In a reaction vial a 250 µl of sample was mixed with 250 µl of ninhydrin solution. This was placed in a boiling water bath for four minutes. A standard solution of arginine was prepared in the range of 0.05-1 mg/ml (0.05, 0.08, 0.10, 0.25, 0.5, 1 mg/ml). Aliquots of 250 µl of each standard was mixed with 250 µl of ninhydrin solution, which were subsequently boiled in a water bath along with the blank and unknown sample for four minutes or until the colour appeared. The absorbance of 200 µl sample mixture was read in an ELISA plate using Multiskan Ascent V1.24 at 550 nm wavelength. A standard curve of the absorbance against arginine concentration was prepared from which subsequently the level of unknown indospicine concentration was determined. This method provided a >70% recovery on 2 mg of arginine dissolved in the loading buffer (0.01 M carbonate buffer pH=10).

### *4.3.4.* ***Statistical analysis***

All studied parameters were subjected to an analysis of variance to investigate the effects of replication, species and accessions nested within a species using proc GLM of SAS (2001). Where F ratio showed significance for species, or accessions within a species, effects, difference between the least squares means were tested using the PDIFF option of SAS (2001), which computes probabilities for all pair wise differences.

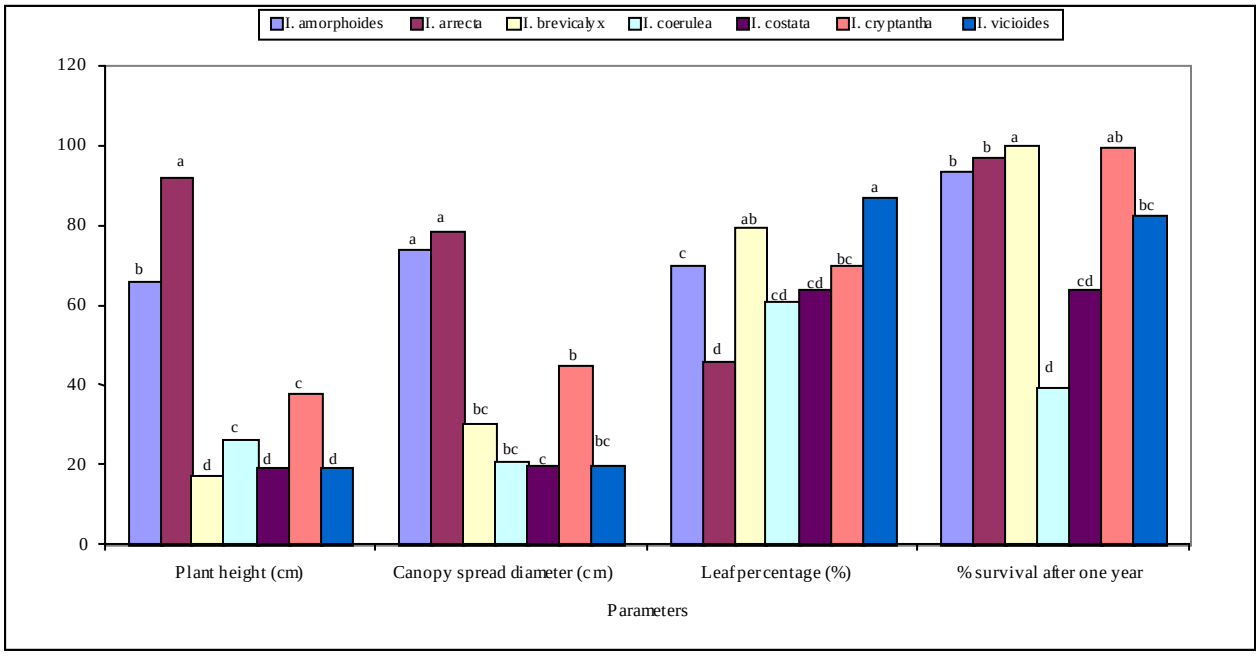
## 4.4. Results

### *4.4.1 Biomass production and winter survival*

Variation between species of *Indigofera* in terms of plant height, canopy spread diameter, total biomass yield and leaf biomass were significant ($P<0.05$) in both the first season (2002/2003) and second season (2003/2004) (Figure 4.1a, 4.1b, 4.2a and 4.2b). In the first season variation between species in terms of average plant height ranged from as low as 17.3 cm (*I. brevicalyx*) to 91.9 cm (*I. arrecta*); canopy spread diameters ranged from 19.7 cm (*I. costata*) to 78.4cm (*I. arrecta*); total biomass yield between 97.2 kg DM/ha (*I. brevicalyx*) to 2728 kg DM/ha (*I. arrecta*) and potentially edible biomass (forage biomass) ranged between 73.9 kg DM/ha (*I. brevicalyx*) to 1150.1 kg DM/ha (*I. arrecta*). The percentage leaf of the total biomass was significantly higher ($P<0.05$) in *I. vicioides* (87.1%) and lowest in *I. arrecta* (45.8%). Similar trends were observed in the second season except that higher values were recorded for *I. arrecta* in terms of agronomic parameters (Figure 4.2a and 4.2b). Seedling survival two months after transplanting and winter survival (after one year) were significantly different ($P <0.05$) between the *Indigofera* species. Generally winter survival was higher for *I. brevicalyx*, *I. arrecta* and *I. cryptantha* followed by *I. brevicalyx* and *I. amorphoides* (Figure 4.1a and 4.2a).

Intra-species differences between collections of accessions were significant ($P<0.05$) in terms of plant height, canopy spread diameter, total biomass, and forage biomass yield. In the 2002/2003 season, variability amongst the accessions of *I. amorphoides* was significant for plant height and forage and total biomass yield (Table 4.1 and 4.2).

a




b

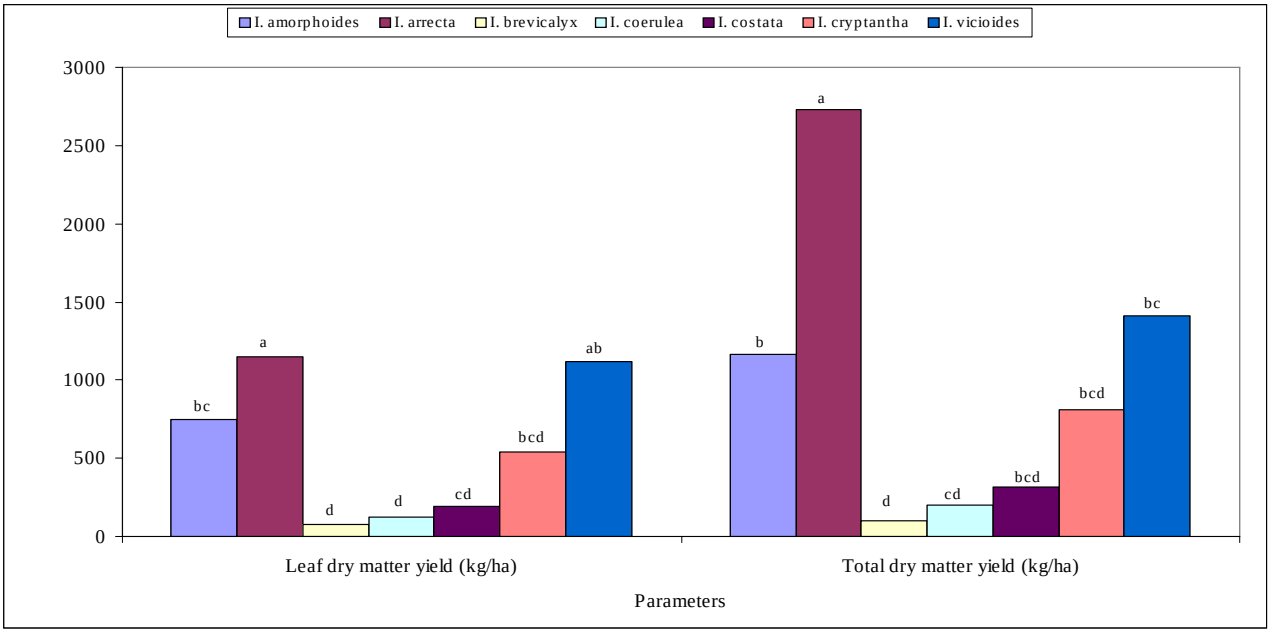
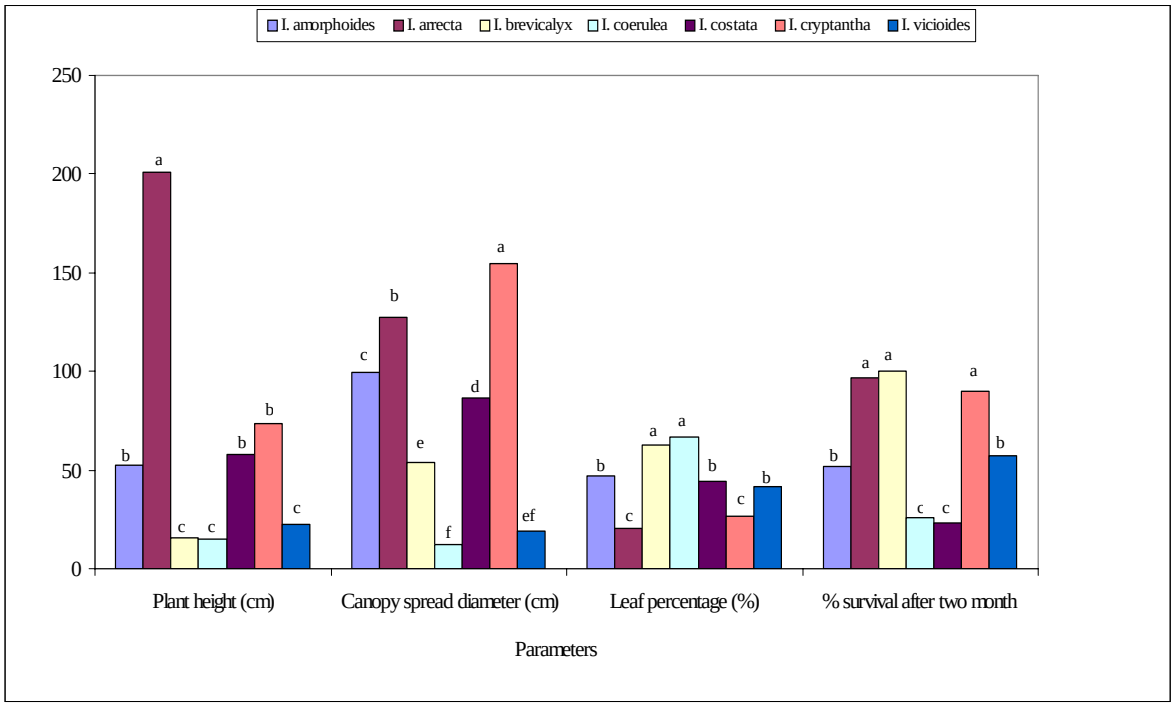



**Figure 4. 1.** Inter-species variations in a collection of *Indigofera* species a) plant height, mean canopy spread diameters, % survival and leaf percentage, and b) leaf dry matter yield and total dry matter production in the first season (2002/2003). Bars for each parameter with different letters differ at P<0.05.

a




b

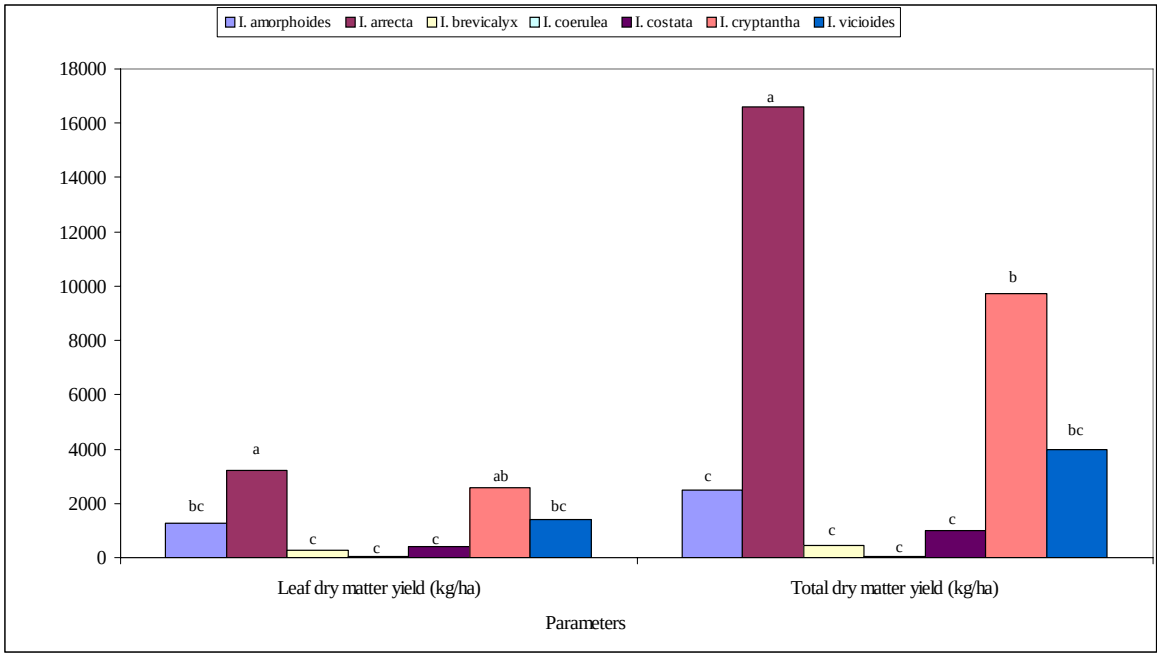



**Figure 4. 2.** Inter-species variations in a collection of *Indigofera* species a) plant height, mean canopy spread diameters, leaf percentage and % survival, and b) leaf dry matter yield and total dry matter production in the second season (2003/2004). Bars for each parameter with different letters differ at P<0.05.

**Table 4. 1.** Intra-species variation in a collection of *Indigofera* species in terms of mean (±s.e.) plant height and canopy spread diameters in the first season (2002/2003).

| Indigofera accessions | Plant height (cm) | Canopy spread diameter (cm) |
|---|---|---|
| *I. amorphoides* 7069 | $64.7^{hijk}$ (±7.16) | $47.3^{ghij}$ (±9.60) |
| *I. amorphoides* 7521 | $50.6^{kl}$ (±7.16) | $54.1^{fghi}$ (±9.60) |
| *I. amorphoides* 7549 | $54.1^{jkl}$(±7.16) | $53.9^{fghi}$ (±9.60) |
| *I. amorphoides* 7557 | $109.5^{cd}$ (±7.16) | $138.0^{a}$ (±9.60) |
| *I. amorphoides* 7570 | $50.8^{kl}$ (±7.16) | $76.2^{cdef}$ (±9.60) |
| *I. arrecta* 7524 | $66.0^{hijk}$ (±7.16) | $55.1^{fghi}$ (±9.60) |
| *I. arrecta* 7592 | $74.0^{fghij}$ (±7.16) | $68.1^{defg}$ (±9.60) |
| *I. arrecta* 7598 | $70.9^{ghijk}$ (±7.16) | $90.1^{cd}$ (±9.60) |
| *I. arrecta* 7709 | $152.7^{a}$ (±7.16) | $68.1^{defg}$ (±9.60) |
| *I. arrecta* 7850 | $100.2^{de}$ (±7.16) | $84.9^{cde}$ (±9.60) |
| *I. arrecta* 8644 | $126.3^{bc}$(±7.16) | $97.1^{bc}$ (±9.60) |
| *I. arrecta* 9045 | $75.4^{fghi}$ (±7.16) | $82.4^{cde}$ (±9.60) |
| *I. arrecta* 10339 | $92.0^{def}$ (±7.16) | $75.6^{cdef}$ (±9.60) |
| *I. arrecta* 10350 | $136.2^{ab}$(±7.16) | $119.1^{ab}$ (±9.60) |
| *I. arrecta* 10355 | $38.8^{lm}$ (±7.16) | $74.3^{cdefg}$ (±9.60) |
| *I. arrecta* 10478 | $87.6^{efg}$ (±7.16) | $65.5^{defg}$ (±9.60) |
| *I. arrecta* 10479 | $83.2^{efgh}$ (±7.16) | $60.8^{efgh}$ (±9.60) |
| *I. brevicalyx* 7815 | $20.4^{mn}$ (±7.16) | $34.2^{hijk}$ (±9.60) |
| *I. brevicalyx* 7848 | $14.3^{n}$ (±7.16) | $25.9^{jk}$ (±9.60) |
| *I. coerulea* 9004 | $26.3^{mn}$ (±7.16) | $20.8^{jk}$ (±9.60) |
| *I. costata* 8712 | $19.2^{mn}$ (±7.16) | $19.7^{k}$ (±9.60) |
| *I. cryptantha* 7067 | $19.5^{mn}$ (±8.84) | $28.^{1ijk}$ (±11.84) |
| *I. cryptantha* 7070 | $56.4^{ijkl}$ (±7.16) | $61.7^{efg}$ (±9.60) |
| *I. vicioides* 10486 | $19.0^{mn}$ (±8.84) | $19.6^{k}$ (±11.84) |

Means within a column followed by different superscript letters differ significantly at $p < 0.05$

**Table 4. 2.** Intra-species variation in a collection of *Indigofera* species in terms of mean (±s.e.) leaf dry matter yield, total dry matter yield and leaf percentage in the first season (2002/2003).

| Indigofera accessions | Leaf dry matter yield (kg/ha) | Total dry matter yield (kg/ha) | Leaf percentage (%) |
|---|---|---|---|
| *I. amorphoides* 7069 | 878$^{cdefghi}$ (±250.8) | 1394$^{efghi}$ (±425.0) | 65.6$^{cde}$ (±4.94) |
| *I. amorphoides* 7521 | 398$^{ghijk}$ (±250.8) | 554$^{hi}$ (±425.0) | 74.8$^{abcd}$ (±4.94) |
| *I. amorphoides* 7549 | 294$^{hijk}$ (±250.8) | 382$^{i}$ (±425.0) | 77.5$^{abc}$ (±4.94) |
| *I. amorphoides* 7557 | 604$^{fghijk}$ (±250.8) | 916$^{ghi}$ (±425.0) | 68.9$^{bcde}$ (±4.94) |
| *I. amorphoides* 7570 | 1558$^{abc}$ (±250.8) | 2578$^{cde}$ (±425.0) | 60.5$^{ef}$ (±4.94) |
| *I. arrecta* 7524 | 984$^{bcdefg}$ (±250.8) | 2122$^{defg}$ (±425.0) | 45.7$^{ghi}$ (±4.94) |
| *I. arrecta* 7592 | 961$^{cdefgh}$ (±250.8) | 2189$^{cdef}$ (±425.0) | 45.3$^{ghi}$ (±4.94) |
| *I. arrecta* 7598 | 1201$^{abcdef}$ (±250.8) | 2944$^{bcd}$ (±425.0) | 41.1$^{hi}$ (±4.94) |
| *I. arrecta* 7709 | 1770$^{a}$ (±250.8) | 5175$^{a}$ (±425.0) | 36.1$^{i}$ (±4.94) |
| *I. arrecta* 7850 | 1680$^{ab}$ (±250.8) | 3358$^{bc}$ (±425.0) | 50.2$^{fgh}$ (±4.94) |
| *I. arrecta* 8644 | 1347$^{abcde}$ (±250.8) | 3824$^{b}$ (±425.0) | 32.5$^{i}$ (±4.94) |
| *I. arrecta* 9045 | 702$^{efghijk}$ (±250.8) | 2019$^{defg}$ (±425.0) | 35.3$^{i}$ (±4.94) |
| *I. arrecta* 10339 | 1421$^{abcd}$ (±250.8) | 3211$^{bcd}$ (±425.0) | 43.8$^{hi}$ (±4.94) |
| *I. arrecta* 10350 | 1419$^{abcd}$ (±250.8) | 4044$^{ab}$ (±425.0) | 35.5$^{i}$ (±4.94) |
| *I. arrecta* 10355 | 678$^{efghijk}$ (±250.8) | 1062$^{fghi}$ (±425.0) | 64.7$^{cde}$ (±4.94) |
| *I. arrecta* 10478 | 725$^{defghij}$ (±250.8) | 1197$^{fghi}$ (±425.0) | 61.5$^{def}$ (±4.94) |
| *I. arrecta* 10479 | 915$^{cdefgh}$ (±250.8) | 1594$^{efgh}$ (±425.0) | 58.1$^{efg}$ (±4.94) |
| *I. brevicalyx* 7815 | 91$^{jk}$ (±250.8) | 121$^{i}$ (±425.0) | 77.2$^{abc}$ (±4.94) |
| *I. brevicalyx* 7848 | 57$^{k}$ (±250.8) | 73$^{i}$ (±425.0) | 80.5$^{ab}$ (±4.94) |
| *I. coerulea* 9004 | 127$^{jk}$ (±250.8) | 203$^{i}$ (±425.0) | 61.1$^{def}$ (±4.94) |
| *I. costata* 8712 | 196$^{ijk}$ (±250.8) | 314$^{i}$ (±425.0) | 63.5$^{cdef}$ (±4.94) |
| *I. cryptantha* 7067 | 278$^{ghijk}$ (±309.4) | 461$^{hi}$ (±524.3) | 70.3$^{abcde}$ (±6.10) |
| *I. cryptantha* 7070 | 795$^{defghij}$ (±250.8) | 1160$^{fghi}$ (±425.0) | 69.2$^{bcde}$ (±4.94) |
| *I. vicioides* 10486 | 1120$^{abcdefg}$ (±309.4) | 1412$^{efghi}$ (±524.3) | 87.1$^{a}$ (±6.10) |

Means within a column followed by different superscript letters differ significantly at $p < 0.05$

*I. amorphoides* 7557 was the tallest among *I. amorphoides* accessions, but 7570 was superior in terms of forage and total biomass yield. Similarly accessions of *I. arrecta* exhibited remarkable variation in terms of plant height, leaf biomass yield and total biomass production (Table 4.1 and 4.2). *I. arrecta* 7709 was superior in terms of plant height (152.7 cm), leaf biomass yield (1770 kg DM/ha) and total biomass production (5175 kg DM/ha). Six other accessions (7850, 7570, 10339, 10350, 8644 and 7598) were similar to this high yielder, while *I. arrecta* 10355 and 9045 were inferior in terms of their edible forage biomass production.

In the 2003/2004 season, intra-species variability between collections of *I. amorphoides* was significant ($P<0.05$) in terms of plant height (Table 4.3) and biomass production (Table 4.4). *I. amorphoides* 7549 was superior in terms of its leaf and total biomass yield (Table 4.4). Variability was also manifest between accessions of *I. arrecta,* which were remarkably high in terms of plant height, canopy spread diameter, edible forage biomass production and total biomass yield (Table 4.3 and 4.4). *I. arrecta* 10478 and 10479 were the tallest with a plant height of 251.6 and 242.8cm, respectively. Once again (in the 2003/2004 season) *I. arrecta* 10350 was superior in terms of total biomass production followed by 7850, 7598, 7592, 9045, 7709, 10479, 8644 and 10339. The accessions exhibited slightly different rankings in this season in terms of edible forage biomass yield, with *I. arrecta* 7850 as the highest yielder followed by 10350, 7067 and 10479 (Table 4.4).

**Table 4. 3.** Intra-species variation in a collection of *Indigofera* species in terms of mean (±s.e.) plant height and canopy spread diameters in the second season (2003/2004).

| Indigofera accessions | Plant height (cm) | Canopy spread diameter (cm) |
|---|---|---|
| *I. amorphoides* 7069 | 81.0$^{f}$ (±11.41) | 102.2$^{cdef}$ (±15.28) |
| *I. amorphoides* 7521 | 52.2$^{fgh}$ (±11.41) | 125.2$^{bcdef}$ (±15.28) |
| *I. amorphoides* 7549 | 80.7$^{f}$ (±11.41) | 107.0$^{bcdef}$ (±15.28) |
| *I. amorphoides* 7557 | 31.0$^{ghi}$ (±11.41) | 42.3$^{hi}$ (±15.28) |
| *I. amorphoides* 7570 | 50.9$^{fgh}$ (±11.41) | 119.8$^{bcdef}$ (±15.28) |
| *I. arrecta* 7524 | 137.4$^{e}$ (±11.41) | 131.9$^{bcd}$ (±15.28) |
| *I. arrecta* 7592 | 186.1$^{cd}$ (±11.41) | 130.7$^{bcde}$ (±15.28) |
| *I. arrecta* 7598 | 159.2$^{de}$ (±11.41) | 142.4$^{abcd}$ (±15.28) |
| *I. arrecta* 7709 | 235.6$^{ab}$ (±11.41) | 87.8$^{efg}$ (±15.28) |
| *I. arrecta* 7850 | 227.8$^{ab}$ (±11.41) | 149.3$^{ab}$ (±15.28) |
| *I. arrecta* 8644 | 227.8$^{ab}$ (±11.41) | 117.2$^{bcdef}$ (±15.28) |
| *I. arrecta* 9045 | 242.2$^{ab}$ (±11.41) | 135.9$^{bcd}$ (±15.28) |
| *I. arrecta* 10339 | 210.0$^{bc}$ (±11.41) | 136.2$^{bcd}$ (±15.28) |
| *I. arrecta* 10350 | 233.4$^{ab}$ (±11.41) | 145.0$^{abc}$ (±15.28) |
| *I. arrecta* 10355 | 59.2$^{fg}$ (±11.41) | 133.1$^{bcd}$ (±15.28) |
| *I. arrecta* 10478 | 251.6$^{a}$ (±11.41) | 119.0$^{bcdef}$ (±15.28) |
| *I. arrecta* 10479 | 242.8$^{a}$ (±11.41) | 99.4$^{def}$ (±15.28) |
| *I. brevicalyx* 7815 | 15.9$^{i}$ (±11.41) | 55.5$^{gh}$ (±15.28) |
| *I. brevicalyx* 7848 | 16.0$^{i}$ (±11.41) | 52.2$^{ghi}$ (±15.28) |
| *I. coerulea* 9004 | 15.0$^{i}$ (±11.41) | 12.2$^{i}$ (±15.28) |
| *I. costata* 8712 | 58.0$^{fg}$ (±11.41) | 86.2$^{fg}$ (±15.28) |
| *I. cryptantha* 7067 | 67.1$^{fg}$ (±14.08) | 188.7$^{a}$ (±18.85) |
| *I. cryptantha* 7070 | 79.7$^{f}$ (±11.41) | 121.0$^{bcdef}$ (±15.28) |
| *I. vicioides* 10486 | 22.8$^{hi}$ (±14.08) | 19.4$^{hi}$ (±18.85) |

Means within a column followed by different superscript letters differ significantly at $p < 0.05$

**Table 4. 4.** Intra-species variation in a collection of *Indigofera* species in terms of mean (±s.e.) leaf dry matter yield, total dry matter yield and leaf percentage in the second season (2003/2004).

| Indigofera accessions | Leaf dry matter yield (kg/ha) | Total dry matter yield (kg/ha) | Leaf percentage (%) |
|---|---|---|---|
| *I. amorphoides* 7069 | 981$^{fghi}$ (±596.8) | 2390$^{efg}$ (±2446.3) | 41.5$^{def}$ (±3.64) |
| *I. amorphoides* 7521 | 1565$^{defghi}$ (±596.8) | 3516$^{efg}$ (±2446.3) | 46.4$^{cde}$ (±3.64) |
| *I. amorphoides* 7549 | 2614$^{bcdef}$ (±737.4) | 4894$^{efg}$ (±3022.6) | 46.3$^{cdef}$ (±4.49) |
| *I. amorphoides* 7557 | 16$^{hi}$ (±737.4) | 32$^{g}$ (±3022.6) | 50.2$^{bcd}$ (±4.49) |
| *I. amorphoides* 7570 | 1099$^{efghi}$ (±596.8) | 2280$^{efg}$ (±2446.3) | 52.1$^{bc}$ (±3.64) |
| *I. arrecta* 7524 | 3201$^{bcd}$ (±596.8) | 13589$^{cd}$ (±2446.3) | 23.5$^{gh}$ (±3.64) |
| *I. arrecta* 7592 | 2774$^{bcde}$ (±596.8) | 19313$^{abc}$ (±2446.3) | 15.5$^{h}$ (±3.64) |
| *I. arrecta* 7598 | 3023$^{bcd}$ (±737.4) | 19322$^{abc}$ (±3022.6) | 17.0$^{gh}$ (±4.49) |
| *I. arrecta* 7709 | 2280$^{bcdef}$ (±596.8) | 17359$^{abc}$ (±2446.3) | 13.3$^{h}$ (±3.64) |
| *I. arrecta* 7850 | 5269$^{a}$ (±596.8) | 21623ab (±2446.3) | 23.4$^{gh}$ (±3.64) |
| *I. arrecta* 8644 | 2780$^{bcde}$ (±596.8) | 15783$^{abc}$ (±2446.3) | 17.7$^{gh}$ (±3.64) |
| *I. arrecta* 9045 | 3119$^{bcd}$ (±596.8) | 18787$^{abc}$ (±2446.3) | 16.0$^{h}$ (±3.64) |
| *I. arrecta* 10339 | 2810$^{bcd}$ (±596.8) | 15578$^{abc}$ (±2446.3) | 18.7$^{gh}$ (±3.64) |
| *I. arrecta* 10350 | 4063$^{ab}$ (±737.4) | 21217$^{a}$ (±3022.6) | 19.0$^{gh}$ (±4.49) |
| *I. arrecta* 10355 | 1937$^{cdefg}$ (±596.8) | 5528$^{efg}$ (±2446.3) | 36.6$^{ef}$ (±3.64) |
| *I. arrecta* 10478 | 3394$^{bc}$ (±596.8) | 15319$^{bc}$ (±2446.3) | 23.0$^{gh}$ (±3.64) |
| *I. arrecta* 10479 | 3658$^{ab}$ (±596.8) | 16030$^{abc}$ (±2446.3) | 22.7$^{gh}$ (±3.64) |
| *I. brevicalyx* 7815 | 260$^{ghi}$ (±596.8) | 399$^{g}$ (±2446.3) | 64.8$^{a}$ (±3.64) |
| *I. brevicalyx* 7848 | 287$^{ghi}$ (±596.8) | 464$^{g}$ (±2446.3) | 61.1$^{ab}$ (±3.64) |
| *I. coerulea* 9004 | 29$^{i}$ (±596.8) | 43$^{g}$ (±2446.3) | 66.7$^{a}$ (±3.64) |
| *I. costata* 8712 | 416$^{ghi}$ (±596.8) | 1013$^{fg}$ (±2446.3) | 43.9$^{cdef}$ (±3.64) |
| *I. cryptantha* 7067 | 3358$^{abcde}$ (±1051.9) | 11915$^{bcde}$ (±4311.8) | 30.9$^{fg}$ (±6.41) |
| *I. cryptantha* 7070 | 1760$^{cdefgh}$ (±596.8) | 7565$^{def}$ (±2446.3) | 22.5$^{gh}$ (±3.64) |
| *I. vicioides* 10486 | 1420$^{defghi}$ (±736.7) | 3981$^{efg}$ (±3020) | 41.5$^{cdef}$ (±4.49) |

Means within a column followed by different superscript letters differ significantly at p <0.05

**Table 4. 5.** Inter-species variation in a collection of *Indigofera* species in terms of nutritive value parameters and indospicine content of leaf dry matter.

| Species | DM (g kg$^{-1}$ DM) | Ash (g kg$^{-1}$ DM) | CP (g kg$^{-1}$ DM) | P (g kg$^{-1}$ DM) | IVOMD (g kg$^{-1}$ DM) | Indospicine (mg kg$^{-1}$ DM) |
|---|---|---|---|---|---|---|
| *I. amorphoides* | 894.9$^{a*}$ (±9.93) | 125.6$^{a}$ (±3.52) | 276.8$^{a}$ (±7.96) | 3.26$^{a}$ (±0.21) | 748.3$^{a}$ (±11.71) | 180.8$^{b}$ (±23.9) |
| *I. arrecta* | 888.5$^{a}$ (±6.39) | 105.3$^{b}$ (±2.23) | 242.7$^{b}$ (±4.94) | 2.83$^{ab}$ (±0.13) | 706.2$^{b}$ (±7.43) | 126.1$^{bc}$ (±15.54) |
| *I. brevicalyx* | 893.8$^{a}$ (±14.18) | 101.9$^{b}$ (±5.02) | 224.3$^{b}$ (±11.37) | 3.50$^{a}$ (±2.94) | 638.2$^{c}$ (±16.72) | 2.0$^{c}$ (±48.85) |
| *I. coerulea* | 904.4$^{a}$ (±24.83) | 129.3$^{a}$ (±8.79) | 159.2$^{c}$ (±19.89) | 2.41$^{ab}$ (±0.51) | 699.2$^{bc}$ (±29.26) | 23.0$^{c}$ (±59.74) |
| *I. costata* | 913.8$^{a}$ (±20.06) | 133.7$^{a}$ (±7.10) | 226.5$^{b}$ (±16.08) | 2.30$^{b}$ (±0.42) | 654.6$^{c}$ (±23.65) | 135.9$^{bc}$ (±48.20) |
| *I. cryptantha* | 909.5$^{a}$ (±20.38) | 90.1$^{b}$ (±7.21) | 298.7$^{a}$ (±16.32) | 3.72$^{a}$ (±0.42) | 736.4$^{ab}$ (±24.02) | 35.4$^{c}$ (±49.07) |
| *I. vicioides* | 909.0$^{a}$ (±35.48) | 111.2$^{ab}$ (±12.56) | 200.6$^{bc}$ (±28.40) | 2.18$^{ab}$ (±0.74) | 609.4$^{c}$ (±41.81) | 705.6$^{a}$ (±85.49) |

$^{*}$ Means within a column followed by different superscript letters differ significantly at $p < 0.05$.

4.4.2 ***Nutritive value***

On average the DM concentration of samples analysed for nutritive value was not significantly different (P>0.05) between the species. However, the species showed significant variations in terms of ash, crude protein (CP), phosphorous (P), *in vitro* organic matter digestibility (IVOMD) and indospicine concentration in the leaves (Table 4.5). Ash content (g $kg^{-1}$ DM) was, in general, lower in *I. cryptantha* (90.1), *I. brevicalyx* (101.9) and *I. arrecta* (105.3) than in *I. amorphoides* (125.6), *I. coerulea* (129.3) or *I. costata* (133.7). The highest level of CP (g $kg^{-1}$ DM) was recorded in *I. cryptantha* (298.7) and *I. amorphoides* (276.8), while the lowest CP level was in *I. coerulea* (159.2) and *I. vicioides* (200.6). The other species were intermediate. Phosphorous (g $kg^{-1}$ DM) of the forage biomass was highest in *I. cryptantha* (3.72), followed by *I. brevicalyx* (3.50) and *I. amorphoides* (3.26), with *I. costata* being the lowest at 2.30. The *in vitro* organic matter digestibility (g $kg^{-1}$ DM) was highest in *I. amorphoides* (748.3) and *I. cryptantha* (736.4) and lowest in *I. brevicalyx* (638.2) and *I. costata* (654.6). The species also exhibited notable variation in terms of indospicine concentrations in the leaves. This ranged, on average, from as low as undetectable levels in *I. brevicalyx* (0 to 2 mg $kg^{-1}$ DM) to as high as 705.6 mg $kg^{-1}$ DM in *I. vicioides*. The levels of indospicine in a species of *I. coerulea* and *I. cryptantha* were low (23 to 35.4 mg $kg^{-1}$ DM), while they were moderate in *I. amorphoides* (180.8 mg $kg^{-1}$ DM), *I. arrecta* (126.1 mg $kg^{-1}$ DM) and *I. costata* (135.9 mg $kg^{-1}$ DM).

Intra-species variations within the *Indigofera* accessions in terms of nutritive value traits were significant (P <0.05) (Table 4.6 and 4.7). In *I. amorphoides* CP level were more or

**Table 4. 6.** Intra-species variation in a collection of *Indigofera* species in terms of mean (±s.e.) ash, CP and P concentration of the leaves in the establishment season (2002/2003).

| Indigofera accessions | Ash (g $kg^{-1}$ DM) | CP (g $kg^{-1}$ DM) | P (g $kg^{-1}$ DM) |
|---|---|---|---|
| *I. amorphoides* 7069 | $118.2^{abcde}$ (±7.10) | $260^{abc}$ (±16.08) | $3.67^{a}$ (±0.416) |
| *I. amorphoides* 7521 | $131.9^{ab}$ (±7.10) | $277^{a}$ (±16.08) | $2.67^{abc}$ (±0.416) |
| *I. amorphoides* 7549 | $111.2^{abcdef}$ (±8.79) | $287^{a}$ (±19.89) | $3.41^{ab}$ (±0.515) |
| *I. amorphoides* 7557 | $132.8^{a}$ (±8.79) | $294^{a}$ (±19.89) | $3.91^{a}$(±0.515) |
| *I. amorphoides* 7570 | $133.6^{a}$ (±7.10) | $266^{abc}$ (±16.08) | $2.63^{abc}$ (±0.416) |
| *I. arrecta* 7524 | $103.5^{def}$ (±7.10) | $185^{ef}$ (±16.08) | $2.00^{c}$ (±0.416) |
| *I. arrecta* 7592 | $119.3^{abcde}$ (±8.79) | $260^{abc}$ (±16.08) | $3.67^{a}$ (±0.416) |
| *I. arrecta* 7598 | $110.3^{bcdef}$ (±8.79) | $253^{abcd}$ (±19.89) | $2.91^{abc}$(±0.515) |
| *I. arrecta* 7709 | $81.6^{gh}$ (±7.10) | $267^{abc}$ (±16.08) | $2.33^{bc}$ (±0.416) |
| *I. arrecta* 7850 | $102.4^{def}$ (±7.10) | $210^{def}$ (±16.08) | $2.33^{bc}$ (±0.416) |
| *I. arrecta* 8644 | $78.0^{h}$ (±7.10) | $225^{cde}$ (±16.08) | $2.67^{abc}$ (±0.416) |
| *I. arrecta* 9045 | $108.8^{cdef}$ (±7.10) | $163^{f}$ (±16.08) | $2.33^{bc}$ (±0.416) |
| *I. arrecta* 10339 | $101.2^{efg}$ (±7.10) | $277^{a}$ (±16.08) | $3.00^{abc}$ (±0.416) |
| *I. arrecta* 10350 | $106.6^{cdef}$ (±8.79) | $225^{bcde}$ (±19.89) | $2.91^{abc}$(±0.515) |
| *I. arrecta* 10355 | $116.2^{abcdef}$ (±7.10) | $296^{a}$ (±16.08) | $4.00^{a}$ (±0.416) |
| *I. arrecta* 10478 | $114.2^{abcdef}$ (±8.79) | $278^{ab}$ (±19.89) | $3.03^{abc}$ (±0.515) |
| *I. arrecta* 10479 | $121.9^{abcd}$ (±7.10) | $273^{ab}$ (±16.08) | $2.77^{abc}$ (±0.416) |
| *I. brevicalyx* 7815 | $107.4^{cdef}$ (±7.10) | $194^{ef}$ (±16.08) | $4.00^{a}$ (±0.416) |
| *I. brevicalyx* 7848 | $96.4^{efgh}$ (±7.10) | $255^{abcd}$ (±16.08) | $3.00^{abc}$ (±0.416) |
| *I. coerulea* 9004 | $129.3^{abc}$ (±8.79) | $159^{f}$ (±19.89) | $2.41^{abc}$ (±0.515) |
| *I. costata* 8712 | $133.7^{a}$ (±7.10) | $226^{cde}$ (±16.08) | $2.30^{bc}$ (±0.416) |
| *I. cryptantha* 7067 | $89.6^{efgh}$ (±12.56) | $301^{a}$ (±28.40) | $4.18^{a}$ (±0.735) |
| *I. cryptantha* 7070 | $90.7^{fgh}$ (±7.10) | $297^{a}$ (±16.08) | $3.27^{ab}$ (±0.416) |
| *I. vicioides* 10486 | $111.2^{abcdef}$ (±12.56) | $201^{cdef}$ (±28.40) | $2.18^{abc}$ (±0.735) |

Means within a column followed by different superscript letters differ significantly at $p < 0.05$

**Table 4. 7.** Intra-species variation in a collection of *Indigofera* species in terms of mean (±s.e.) IVDOM and indospicine concentration of the leaves in the establishment season (2002/2003).

| Indigofera accessions | IVDOM (g $kg^{-1}$ DM) | Indospicine (mg $kg^{-1}$ DM) |
|---|---|---|
| *I. amorphoides* 7069 | $800^{ab}$ (±23.6) | $194^{bcde}$ (±48.2) |
| *I. amorphoides* 7521 | $801^{a}$ (±23.6) | $314^{b}$ (±48.2) |
| *I. amorphoides* 7549 | $727^{abcd}$ (±29.2) | $126^{defg}$ (±59.7) |
| *I. amorphoides* 7557 | $697^{cdef}$ (±29.2) | $146^{cdefg}$ (±59.7) |
| *I. amorphoides* 7570 | $717^{cd}$ (±23.6) | $124^{efg}$ (±48.2) |
| *I. arrecta* 7524 | $692^{cdef}$ (±23.6) | $29^{fg}$ (±48.2) |
| *I. arrecta* 7592 | $720^{cd}$ (±29.2) | $41^{fg}$ (±48.2) |
| *I. arrecta* 7598 | $724^{bcd}$ (±29.2) | $60^{efg}$ (±59.7) |
| *I. arrecta* 7709 | $650^{def}$ (±23.6) | $289^{bc}$ (±48.2) |
| *I. arrecta* 7850 | $654^{def}$ (±23.6) | $26^{fg}$ (±48.2) |
| *I. arrecta* 8644 | $704^{cde}$ (±23.6) | $268^{bcd}$ (±48.2) |
| *I. arrecta* 9045 | $722^{cd}$ (±23.6) | $46^{fg}$ (±48.2) |
| *I. arrecta* 10339 | $706^{cde}$ (±23.6) | $217^{bcde}$ (±59.7) |
| *I. arrecta* 10350 | $698^{cdef}$ (±29.2) | $108^{efg}$ (±59.7) |
| *I. arrecta* 10355 | $755^{abc}$ (±23.6) | $56^{efg}$ (±59.7) |
| *I. arrecta* 10478 | $748^{abc}$ (±29.2) | $174^{bcdef}$ (±59.6) |
| *I. arrecta* 10479 | $702^{cdef}$ (±23.6) | $198^{bcde}$ (±48.2) |
| *I. brevicalyx* 7815 | $622^{f}$ (±23.6) | $8.8^{g}$ (±48.2) |
| *I. brevicalyx* 7848 | $655^{def}$ (±23.6) | $0^{fg}$ (±85.0) |
| *I. coerulea* 9004 | $699^{cde}$ (±29.2) | $23^{fg}$ (±59.7) |
| *I. costata* 8712 | $655^{def}$ (±23.6) | $136^{defg}$ (±48.2) |
| *I. cryptantha* 7067 | $766^{abc}$ (±41.8) | $6^{fg}$ (±85.5) |
| *I. cryptantha* 7070 | $707^{cde}$ (±23.6) | $65^{efg}$ (±48.2) |
| *I. vicioides* 10486 | $609^{ef}$ (±41.8) | $706^{a}$ (±85.5) |

Means within a column followed by different superscript letters differ significantly at $p < 0.05$

less similar, while the *in vitro* digestibility varied in the range of 697 g $kg^{-1}$ DM (7557) to 801 g $kg^{-1}$ DM (7521) and indospicine between 124 mg $kg^{-1}$ DM (7570) and 314 mg $kg^{-1}$ DM (7521). The two accessions of *I. brevicalyx* exhibited variability in terms of CP content, while the two accessions of *I. cryptantha* were similar in terms of CP, P, *in vitro* organic matter digestibility and indospicine content of the forage material. Differences between accessions of *I. arrecta* in terms of CP, P, IVOMD, and indospicine level were significant ($P < 0.05$) and remarkably high for some of the parameters (Table 4.6 and 4.7). All accessions of *I. arrecta*, except 7524 and 9045, had CP contents of more than 200 g $kg^{-1}$ DM, with the lowest values being observed in *I. arrecta* 9045 and 7524. The *in vitro* digestibility of organic matter (OM) also varied slightly between accessions of *I. arrecta*. Lower digestibility values (650 g $kg^{-1}$ DM) were recorded in 7709 and 7850, with the highest digestibility value (750 g $kg^{-1}$ DM) being observed in 10355 and 10478. Six accessions of *I. arrecta* (7850, 7524, 7592, 9045, 10355 and 7598) had low levels of indospicine (26.2 to 60 mg $kg^{-1}$ DM). In contrast, four other accessions (7709, 8644, 10339 and 10479) had levels as high as 198 to 289 mg $kg^{-1}$ DM, with the remaining two accessions (10350 and 10478) being intermediate in terms of their indospicine content.

### 4.5. Discussion

A great deal of diversity in forage production potential was demonstrated both within and between the *Indigofera* species. Among the species included in this study *I. arrecta*, *I. vicioides*, *I. amorphoides* and *I. cryptantha*, in decreasing order, demonstrated relatively high forage yield potential in the establishment season, whereas the forage yield potentials of *I. costata*, *I. coerulea* and *I. brevicalyx* were generally inferior.

The *Indigofera* species included in the present study exhibited notable variation both between and within species and had great potential in terms of nutritive value of their forage. The leaves contained medium to high levels of CP (159.2-298.7 g $kg^{-1}$ DM). NRC (1985; 1989) suggested that the diet for mature beef cattle should contain a minimum of 70 g $kg^{-1}$ DM CP, while that for high producing dairy cows was 190 g $kg^{-1}$ DM CP. Almost all these *Indigofera* species could, therefore, be used to supplement low quality roughages for beef animals, while most of the species, except *I. coerulea* 9004 and *I. arrecta* 9045, will practically satisfy, as sole diet, the CP requirement of high producing dairy cows. There seems to be a pattern in relating the CP concentrations of accessions against the collection site environment. Most of the *Indigofera* accessions with lower CP concentration originated from the lowland areas or from mid altitude area reciviving relatively low rainfall.

The CP levels of *Indigofera* accessions were generally higher than browse species, such as *Flemingia macrophylla* (Dzowela *et al*. 1995), *Acacia nilotica, Albizia lebbeck*, *Butea monosperma* (Ramana *et al*. 2000), *Vernonia amygdalina* (El hassen *et al*. 2000), *Cassia sturtii* (Van Niekerk *et al*. 2004; Wilcock *et al*. 2004; Ventura *et al*. 2004), *Rumex linaria*, *Acacia salicina*, *Adenocorpus foliosus* (Ventura *et al*. 2004), while, they were comparable to *Cajanus cajan*, *Acacia angustissima*, *Callindra calothyrsus*, *Gliricidia sepium* and S*esbania macrantha* (Dzowela *et al*. 1995), *Leucaena leucocephala*, *Pongamia pinnata* (Ramana *et al*. 2000), *Medicago sativa*, *Sesbania sesban* (El hassen *et al*. 2000), *Atriplex nummularia* (Van Niekerk *et al*. 2004), *Sutherlanda microphylla*,

*Tripteris sinuatum* (Wilcock *et al*. 2004), *Bituminaria bituminosa* (Ventura *et al*. 2004). Although the high CP levels may indicate a high nutritive value of *Indigofera* species, compared to most browse species, the presence of indospicine in large quantities in some of the species, or accessions, along with other plant nitrogenous secondary metabolites could result in an over estimation of their nutritive value.

The phosphorous content of the forage biomass was higher than that reported for *Acacia* species (Abdulrezak *et al.* 2000), and higher than the lowest level (2g $kg^{-1}$ DM) recommended to meet growth requirements of cattle (ARC, 1980). According to McDowell (2005), the critical level of P concentration recommended to meet the requirements of ruminants was slightly on the higher side, i.e. 2.5 g P $kg^{-1}$ DM, as compared to the ARC (1980) recommendation. The *in vitro* OM digestibility of the poorer species was still 650 g $kg^{-1}$ DM, while it was as high as 800 g $kg^{-1}$ DM for the best. This was within and above the range reported for tropical browse plants (up to 690 g $kg^{-1}$ DM) by Sawe *et al*. (1998) and higher than the figures reported by Aganga *et al*. (2003) for *Atriplex nummalaria* and *Atriplex canescens;* Ventura *et al.* (2004) for *Bituminaria bituminosa, Rumex linaria, Acacia salicina* and *Adenocorpus foliosus* or Wilcock *et al.* (2004) for *Cassia sturtii.* It is, however, comparable with accessions of *Tripteris sinuatum, Sutherlandia microphylla, Atriplex canescens, A. halimus* and *A. nummularia* (Wilcock *et al*. 2004; Van Niekerk *et al*. 2004).

Differences in chemical composition have generally a strong bearing on the potential use of the leguminous multipurpose fodder trees in feeding systems (Dzowela *et al*. 1997), as they may affect palatability and intake by livestock both within and between species and

provenances. In *Indigofera* species secondary plant metabolites could probably influence palatability and intake but the level of indospicine is a better indicator of the potential toxicity of the feed under examination. Both inter- and intra-species variation have been demonstrated for *Indigofera* accessions in terms of indospicine content of leaves, which were in the range of low to high (2 to 750.6 mg $kg^{-1}$ DM). In this study the concentrations of indospicine recorded for most accessions (*I. brevicalyx, I. coerulea, I. cryptantha, I. arrecta, I. costata* and *I. amorphoides*) were lower than the level reported for *I. volkensii* CPI No 33819 (2000 mg $kg^{-1}$ DM) and 33 different *I. spicata* (500 to 12000 mg $kg^{-1}$ DM) accessions (Aylward *et al*. 1987). However, the threshold level, detrimental to animals, has not been precisely determined, though in *I. nigritana* (CPI No. 89268) concentrations as low as 100 mg $kg^{-1}$ DM have resulted in incipient liver lesions (Aylward *et al.* 1987). The same authors reported variability between accessions in terms of toxicity, in a rat bioassay study. Out of 46 accessions tested 13 accessions, from seven species, were considered to be non-toxic while all accessions of *I. spicata* depressed live weight gain and caused varying degrees of liver damage in rats (Aylward *et al.* 1987). Most of the accessions originating from highland areas, or mid altitude areas receiving a high rainfall, were higher in terms of the indospicine concentration in the forage.

The data presented on biomass yields, winter survival, CP, *in vitro* digestibility and indospicine levels have demonstrated that some of the *Indigofera* species/accessions under evaluation, have moderate to high biomass yields, a high crude protein concentration, a high digestibility and low indospicine concentration in the leaves (e.g. 7850, 7598, 7592, etc). This makes them potential candidates for use as protein

supplements. However, chemical composition alone will have a limited value in predicting the nutritive value of a new feed, which may contain materials toxic to the animal. The presence of indospicine in some of the species and/or accessions in relatively large quantities (e.g. 7709, 10339, 8644, 10350, etc) may be a major constraint to their efficient utilization by the animal. Future research needs to address how this may be overcome, if *Indigofera* species are to be used widely as forage plants. On the other hand, the remarkable variability observed in this study, both between and within species in terms of CP, IVOMD and indospicine concentration, suggests the possibility of directly selecting accessions with high forage potential and feeding value for subsequent evaluation with target animals.

# CHAPTER 5

# Variation in growth, dry matter yield and allocation, water use and water use efficiency response of four *Indigofera* species subjected to moisture stress and non-stress conditions

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## 5.1. Abstract

The effects of moisture stress on growth, dry matter accumulation and allocation, water use and water use efficiency were evaluated in four *Indigofera* species (*I. amorphoides*, *I. arrecta*, *I. coerulea* and *I. vicioides*) using a pot experiment under glasshouse conditions. Based on plant available water (PAW) levels, three moisture levels were applied (no stress or 70-100% PAW, 40-70 % PAW and 10-40 % PAW) as experimental treatments and imposed on each species in a completely randomised design with four replications. Moisture stress significantly reduced ($P < 0.05$) the total leaf area of *I. amorphoides* at moderate levels and that of *I. vicioides* at the most severe moisture stress level. The relative growth rate was significantly affected ($P < 0.05$) in *I. amorphoides* but not in *I. arrecta*, *I. coerulea* or *I. vicioides* plants subjected to moisture deficit stress. The dry matter yields of *I. arrecta*, *I. coerulea* and *I. vicioides* were not, however, affected ($P > 0.05$) by moisture stress. Drought stress tolerance indices were not different ($P > 0.05$) for *I. arrecta* or *I. coerulea* grown under no stress and moisture deficit stress conditions. The total biomass yield of *I amorphoides* was, however, reduced due to the effect of moisture stress in reducing both leaf area and leaf area ratio. The root mass fractions of *I. amorphoides* and *I. arrecta* were substantially increased ($P < 0.05$) due to moisture stress. Water use efficiency was low in *I. amorphoides*, under water deficit conditions, while that of *I. vicioides* was higher under severe stress conditions than under non-stress conditions. Transpiration efficiency was, however, higher at moderate moisture deficit stress levels than under non-stressed or severely stressed plants. Generally, the species investigated exhibited significant variation in terms of their response to moisture deficit stress. *I amorphoides* was relatively sensitive while *I. vicioides* was able to maintain unabated growth under water stress conditions. This is highly relevant to programmes aimed at identifying suitable species as a source of fodder for livestock exposed to long dry seasons and frequent drought phenomena.

**Keywords:** dry matter yield, growth, *Indigofera*, moisture stress

## 5.2. Introduction

Water is a scarce resource that determines the growth of rangelands in ecological areas with distinct dry seasons such as savannah and grasslands of the wet- and-dry tropics of Africa. In these areas forage production is mainly limited by moisture deficit stress, and this has a direct impact on the capacity and efficiency of the photosynthetic apparatus (canopy) and consequently on the amount of radiation that could be intercepted and utilized (Monteith 1977; Squire 1990). Supplemental irrigation might increase biomass production, but irrigation is not available to agro-pastoral and pastoral farmers, residing in such areas. The selection of forage species/ecotypes from the native flora, which have higher water use efficiency, could be more appropriate, beneficial and sustainable. *Indigofera* species are among the useful flora that have a good potential as forage and/or cover crops. Naturally the *Indigofera* species are distributed across a wide range of agro-ecological areas, which ranges from arid to sub-humid conditions. Periods of water stress of varying length and severity are a feature of the environment to which the majority of the species are exposed (Hassen 2006, Unpublished data). Consequently, leaf biomass yield and water use efficiency (WUE) for edible or total biomass production is likely to vary between plants of the same species or different species. It is this difference in water use efficiency that confers ecological advantage to the more efficient species (Pearson and Ison 1997). In particular, those species with mechanisms that maintain plant persistency and leaf production through periods of dry season or moisture deficit are likely to be important (Turner and Begg 1978) as pasture plants for sustainable improvement of grazing resources in semi-arid and arid areas.

However, the assessment of genetic variation in water use efficiency, either between or within a species, demands the accurate determination of both transpiration and total biomass production. In forage plants, where leaf yield or edible biomass is the main economic trait, WUE can be calculated as a given level of edible biomass or leaf yield per unit of water used by the plants. In other words, fodder yield is a product of water use (WU), WUE and leaf percentage (LP) of the forage plant. Similarly, the amount of water used can be described in gross terms as evapo-transpiration (ET), which includes transpiration and evaporation, or only as transpiration (T). There are, to date, no available reports on the effect of water stress on the growth, biomass accumulation and forage quality of *Indigofera* species.

The primary objective of this study was to examine variation in biomass accumulation, water use and transpiration efficiency among four *Indigofera* species, that have potential value as forages, when subjected to simulated moisture stress and non-stress conditions.

**5.3. Materials and Methods**

Four *Indigofera* accessions representing four different species (*Indigofera amorphoides* 7570, *Indigofera arrecta* 7524, *Indigofera coerulea* 9004 and *Indigofera vicioides* 10486) were planted, each in 24 pots, in the glasshouse in a randomised complete block design with four replications. The plants were grown at an average of 30 and 20 ºC day and night temperature, respectively. Each accession was planted in 3.04 kg of air-dry soil composed of a mixture of 50% sand and 50% compost. Up to five seeds were sown in each pot. Seedlings were then thinned to two per pot at the two weeks stage. The air-dry

moisture content of the soil was determined as 20.9% of field capacity (FC), and 5.1% of permanent wilting point (PWP), gravimetrically. The volume of water held between FC and PWP was considered as plant available water (PAW). Under field conditions this is the volume of water held between field capacity and wilting point with energy of between –0.03 and –1.5 MPa, in the root zone (Pearson and Ison 1997).

The stress treatments were imposed from the age of 5 weeks after planting. Three moisture levels were applied for each species, to represent three different levels of PAW ranges in the different pots. The first treatment was maintained at 70-100 % PAW (no stress), while the other two were maintained at moderate (40-70 % PAW) or severe (10-40 % PAW) moisture deficit stresses. Once a day pots were replenished with the amount of water equivalent to the loss in weight to bring them to the predetermined level of moisture, whenever the weight of the pots fell to the lower limit established for each treatment. The moisture levels were, therefore, about 5.02-36.4%, 36.4-67.8% and 67.8-99.2% of available soil moisture for the severely stressed, moderately stressed and control treatments, respectively. Due to the increase in the size of control plants, (as the trial progressed) watering was adjusted to twice a day to ensure that control plants showed little or no signs of water stress. The amount of water evaporated (Es) from each treatment was monitored daily by weighing unplanted pots placed between planted pots in both the stressed and non-stressed treatments in each block (three in each block). The amount of water transpired was determined by subtracting the weight loss of planted pots, due to evapo-transpiration (ET), from the weight loss of unplanted pots due to soil evaporation (Es). The latter was determined separately, within a block, for each treatment

level. The pots were equally spaced within a block, with the positions of pots being changed every week, to ensure equal exposure to the growing conditions in the glasshouse.

Four randomly selected pots, each with two plants, grown under the same conditions, for each accession, were destructively harvested to determine the initial number of leaves per plant, mean area per leaf, total green leaf area, total biomass yield and proportion of biomass allocated to the different plant parts (leaf, stem, root, etc.). This was undertaken immediately before the imposition of stress treatments and again at the end of the experiment, after 21 days of treatment. Two plants per pot were maintained throughout the experiment and these were harvested and oven dried at 70 º C for the determination of moisture percentage. Green leaf area was measured with a portable CI-202 leaf area meter (CID Inc., Vancouver, Washington State, USA). Mean relative leaf area expansion rate was estimated as the slope of the natural logarithm of the leaf area versus time. Total biomass and component (leaf, stem and root) yields were determined as the average of the two plants.

Classical plant growth analysis was calculated across one harvest interval as described by Hunt (1982), Gardner *et al.* (1985) and Poorter *et al.* (1989) to estimate mean values for selected growth parameters (relative growth rate, nett assimilation rate, specific leaf area, leaf weight fraction, leaf area ratio, etc.) of individual plants. Specific leaf weight (SLW) expressed, as g $cm^{-2}$ was determined as an average of the ratio of leaf weight versus leaf area measured at two points. Specific leaf area (SLA), expressed as $cm^2$ $g^{-1,}$ was

measured as a mean of a ratio of leaf area versus leaf weight measurements at two points. Relative leaf-area expansion rate, expressed as $cm^2cm^{-2}$ $day^{-1}$, was determined as the slope of the natural logarithm of total leaf area versus time. Relative growth rate was calculated for total biomass dry matter (RGR) as the slope of the natural logarithm of total biomass dry matter versus time, respectively. Leaf area ratio (LAR) was calculated as the average of the ratio of total leaf area over total biomass as measured at two points. Nett assimilation rate (NAR) expressed as in mg $g^{-1}$ $day^{-1}$ was calculated as follows:

$$NAR= [(W_2-W_1)/(T_2-T_1)] * [(InLA_2-InLA_1)/(LA_2-LA_1)] *1\ 000$$ (Gardner *et al.* 1985)

Where, $W_1$ and $W_2$ are total biomass measured at $T_1$ and $T_2$, respectively

$LA_1$ and $LA_2$ are the leaf areas of the plant measured at time $T_1$ and $T_2$ respectively.

Drought stress tolerance indices (DSTI) were computed as a function of leaf ($DSTI_{LDM}$), or total biomass yield ($DSTI_{TDM}$), and these were calculated as a ratio of stressed plant leaf or total biomass yield over control plants leaf or total biomass yield, respectively. Similarly water stress indices (WSI) were computed as a function of ET ($WSI_{ET}$) or T ($WSI_T$), and these were calculated as a ratio of ET, or T, from stressed plants over the ET or T of control plants, respectively. The control plant ET, or T, value approximately represented the potential evapo-transpiration (PET) or potential transpiration (PT), respectively.

Cumulative water use (ET) was obtained from the summation of water applied over the entire study period. Transpiration (T) was calculated from the difference between WU and soil evaporation (Es). Both ET and T were expressed as kg/plant. Water use efficiency (WUE), was determined as a function of nett leaf biomass yield ($WUE_{LDM}$) and nett total dry matter yield ($WUE_{TDM}$). The respective yields were divided by the cumulative amount of water lost through evapo-transpiration (kg/plant). Similarly transpiration efficiency (TE) was also expressed on the basis of leaf dry matter yield ($T_{LDM}$) or total biomass yield ($T_{TDM}$), per kg of water lost through transpiration of the plants.

All studied parameters were subjected to analysis of variance to investigate the effects of moisture level on each species separately using proc GLM of SAS (2001). Where the F ratio showed significance for treatment effect, differences between the least squares means were tested using the PDIFF option of SAS (2001), which computes probabilities for all pair wise differences.

## 5.4. Results

### *5.4.1 Leaf area*

The effect of moisture deficit stress on leaf area of the four species is presented in Table 5.1. Total leaf area per plant was reduced at the moderate stress level for *I. amorphoides* and at the severe stress level for *I. vicioides*, while it was not significantly affected by moisture stress in *I. arrecta* and *I coerulea*. The mean area per leaf was not significantly affected by moisture stress for any of the species. While the leaf number was reduced

**Table 5. 1.** Mean values of some canopy attributes of four *Indigofera* species grown under stressed and non-stressed conditions

| | | Leaf parameters | | | |
|---|---|---|---|---|---|
| Species | Moisture level | Mean area per leaf ($cm^2$ $leaf^{-1}$) | Mean leaf number per plant | Total leaf area ($cm^2$ $plant^{-1}$) | Relative leaf area expansion rate ($cm^2$ $cm^{-2}$ $day^{-1}$) |
| *I.amorphoides* | Control | 10.6 | 95.0a | 997.6a | 0.011a |
| | Moderate stress | 8.5 | 63.2ab | 511.7b | -0.021b |
| | Severe stress | 8.7 | 43.7b | 356.3b | -0.039c |
| | SE | 1.24 | 8.18 | 51.09 | 0.0034 |
| *I.arrecta* | Control | 5.2 | 106.4 | 614.2 | 0.014 |
| | Moderately stress | 5.8 | 98.3 | 552.5 | 0.023 |
| | Severely stress | 3.6 | 92.3 | 330.5 | -0.002 |
| | SE | 0.84 | 14.17 | 128.45 | 0.0156 |
| *I.coerulea* | Control | 21.1 | 9.7 | 200.8 | 0.044 |
| | Moderate stress | 17.3 | 7.0 | 129.3 | 0.020 |
| | Severe stress | 16.8 | 7.1 | 122.3 | 0.022 |
| | SE | 3.35 | 1.05 | 45.92 | 0.0138 |
| *I.vicioides* | Control | 4.9 | 84.7 | 370.9a | 0.126a |
| | Moderate stress | 5.3 | 59.3 | 309.8ab | 0.117a |
| | Severe stress | 4.1 | 45.9 | 185.8b | 0.093b |
| | SE | 0.52 | 14.13 | 35.91 | 0.0050 |

Means with different letters between moisture levels within species differ at $P < 0.05$.

(P<0.05) in *I. amorphoide* due to severe stress, it was not affected in the other species. The relative leaf area expansion rate was significantly reduced at the moderate stress level (P <0.05) for *I amorphoides*, and this was reduced further with an increasing level of stress in *I amorphoides*, whereas, in *I vicioides* the leaf area expansion rate was only significantly affected at the severe stress level. The leaf area expansion rates of *I. arrecta* and *I. coerulea* were not significantly affected (P >0.05) by moisture stress.

5.4.2. ***Dry matter yield, dry matter allocation and plant growth***

Severe moisture stress significantly reduced (P <0.05) the total biomass yield of *I. amorphoides*, while stress has no significant effect (P >0.05) on the total biomass yields of *I. arrecta*, *I. coerulea* or *I. vicioides* (Table 5.2). The stem and root biomass yields of *I. amorphoides* were significantly reduced (p <0.05) by moderate stress level, while they were not affected in the other three species. Leaf biomass yields tended to decline with increasing stress, but the reduction was only statistically significant in *I. vicioides*.

Severe moisture stress significantly reduced (P <0.05) the relative growth rate (RGR) and leaf area ratio (LAR) in *I. amorphoides*, while neither of these parameters were affected by stress in *I arrecta*, *I. coerulea* or *I. vicioides* (Table 5.3). The nett assimilation rate (NAR), specific leaf weight (SLW) and specific leaf area (SLA) were not significantly affected (P >0.05) by stress in any of the species. The dry matter allocation patterns of the species, however, varied. Leaf mass fraction (LMF) tended to decline with increase in stress in all the species, though these differences were not statistically significant. Stem

**Table 5. 2.** Dry matter yields and stress tolerance indices of four *Indigofera* species grown under stressed and non-stressed conditions.

| Species | Moisture level | DM yield (g plant$^{-1}$) | | | | Stress tolerance index | |
|---|---|---|---|---|---|---|---|
| | | Leaf mass | Stem mass | Root mass | Total biomass | $DSTI_{LDM}$ | $DSTI_{TDM}$ |
| *I.amorphoides* | Control | 5.1 | 3.4a | 3.8a | 10.3a | 1.569 | 1.620a |
| | Moderate stress | 3.5 | 2.3b | 3.2b | 7.0ab | 1.318 | 1.363ab |
| | Severe stress | 2.6 | 2.1b | 3.2b | 5.9b | 1.176 | 1.275b |
| | SE | 0.60 | 0.16 | 0.10 | 0.80 | 0.0950 | 0.0629 |
| *I.arrecta* | Control | 3.8 | 2.6 | 2.5 | 6.9 | 1.456 | 1.453 |
| | Moderate stress | 3.6 | 2.6 | 3.0 | 7.2 | 1.408 | 1.485 |
| | Severely stress | 2.6 | 2.1 | 2.7 | 5.4 | 1.229 | 1.310 |
| | SE | 0.67 | 0.37 | 0.36 | 1.38 | 0.1279 | 0.1304 |
| *I.coerulea* | Control | 1.6 | 1.2 | 1.2 | 1.9 | 1.611 | 1.568 |
| | Moderate stress | 1.4 | 1.1 | 1.1 | 1.6 | 1.412 | 1.365 |
| | Severe stress | 1.3 | 1.1 | 1.1 | 1.5 | 1.348 | 1.323 |
| | SE | 0.17 | 0.07 | 0.05 | 0.27 | 0.1689 | 0.1658 |
| *I.vicioides* | Control | 2.5a | 1.7 | 1.3 | 3.5 | 1.742a | 1.688 |
| | Moderate stress | 2.2ab | 1.7 | 1.3 | 3.2 | 1.601ab | 1.595 |
| | Severe stress | 1.8b | 1.5 | 1.3 | 2.5 | 1.370b | 1.388 |
| | SE | 0.15 | 0.11 | 0.05 | 0.26 | 0.0771 | 0.0798 |

Means with different letters between moisture levels within species differ at $P < 0.05$.

**Table 5. 3.** Plant growth parameters and biomass allocation pattern of four *Indigofera* species grown under moisture stressed and non-stressed conditions.

| Species | Moisture level | RGR* | LAR | NAR | SLW | SLA | LMF | SMF | RMF |
|---|---|---|---|---|---|---|---|---|---|
| *I.amorphoides* | Control | 49.5a[1] | 176.9a | 0.123 | 4.1 | 372.4 | 0.420 | 0.260 | 0.318b |
| | Moderate stress | 30.7ab | 163.6ab | 0.059 | 5.2 | 310.7 | 0.428 | 0.220 | 0.355ab |
| | Severe stress | 21.2b | 157.4b | -0.009 | 5.0 | 315.3 | 0.345 | 0.220 | 0.435a |
| | SE | 4.37 | 3.61 | 0.0302 | 0.51 | 33.16 | 0.0386 | 0.0169 | 0.0230 |
| *I.arrecta* | Control | 31.7 | 115.9 | 0.098 | 4.9 | 228.4 | 0.455 | 0.278 | 0.268b |
| | Moderate stress | 42.6 | 110.1 | 0.140 | 4.9 | 226.1 | 0.423 | 0.253 | 0.325ab |
| | Severely stress | 26.2 | 103.3 | 0.048 | 5.3 | 218.0 | 0.388 | 0.245 | 0.370a |
| | SE | 12.52 | 5.44 | 0.0727 | 0.17 | 4.56 | 0.0382 | 0.0262 | 0.0189 |
| *I.coerulea* | Control | 43.3 | 180.3 | 0.133 | 3.5 | 260.7 | 0.638 | 0.193a | 0.170 |
| | Moderate stress | 20.5 | 180.2 | 0.061 | 3.7 | 259.0 | 0.643 | 0.155ab | 0.200 |
| | Severe stress | 23.5 | 177.5 | 0.056 | 3.6 | 263.7 | 0.608 | 0.133b | 0.260 |
| | SE | 16.19 | 6.77 | 0.0621 | 0.25 | 9.78 | 0.0145 | 0.0121 | 0.0235 |
| *I.vicioides* | Control | 121.1 | 133.7 | 0.515 | 4.3 | 199.8 | 0.595 | 0.290 | 0.115 |
| | Moderate stress | 115.8 | 128.9 | 0.477 | 4.3 | 199.7 | 0.553 | 0.290 | 0.158 |
| | Severe stress | 98.4 | 122.1 | 0.419 | 4.8 | 191.0 | 0.533 | 0.288 | 0.178 |
| | SE | 5.70 | 4.25 | 0.0338 | 0.27 | 7.00 | 0.0185 | 0.0171 | 0.0197 |

Means with different letters between moisture levels within species differ at P <0.05.

* RGR=relative growth rate (mg $g^{-1}$ $day^{-1}$); LAR= leaf area ratio ($cm^2$ $g^{-1}$); NAR= Nett assimilation rate (mg $cm^{-2}$ $day^{-1}$); SLW=Specific leaf weight (g $cm^{-2}$); SLA=Specific leaf area ($cm^2$ $g^{-1}$); LMF= leaf mass fraction; SMF= Stem mass fraction; RTF= Root mass fraction.

mass fractions (SMF) were significantly reduced (P <0.05) by severe stress in *I. coerulea*, but not in the other species. However, the proportion of dry matter allocated to the roots was significantly increased (P <0.05) in *I. amorphoides* and *I. arrecta*, but not in *I. coerulea* and *I. vicioides* (Table 5.3).

### 5.4.3. *Plant water stress index and drought stress tolerance index*

Plant water stress indices, based on water use, were significantly different between the three moisture levels in all species (Table 5.4). However, plant water stress indices, based on transpiration, responded variably between the species. Plant water stress was detected at the moderate level of stress in *I amorphoides* and *I. coerulea*, while it was only detected under severe stress in *I. arrecta* and not detected at all in *I. vicioides* (Table 5.4).

The *Indigofera* species exhibited variation in terms of drought stress tolerance indices, calculated on the basis of leaf biomass or total biomass production. Stress tolerance indices calculated on the basis of leaf biomass yield were unaffected (P >0.05) by moisture levels in *I. amorphoides, I. arrecta* and *I. coerulea* while drought stress tolerance indices, based on leaf biomass, exhibited significant decreases (P <0.05) in *I. vicioides* under severe stress. In contrast, the plant stress tolerance index, calculated in terms of total biomass yield, was significantly reduced (P <0.05) in *I. amorphoides* at severe stress level, while the plant stress tolerance index, in terms of total biomass yield, was not affected (P >0.05) at all in *I. arrecta, I. coerulea or I. vicioides* (Table 5.4).

**Table 5. 4.** Cumulative water use, water stress indices based on evapo-transpiration and water use efficiencies based on leaf dry matter yield and total biomass of four *Indigofera* species grown under moisture stressed and non-stressed conditions.

| Species | Moisture level | Cumulative water Use ($kg.plant^{-1}$) | Water stress index ($WSI_{ET}$) (1-ET/PET) | Water use efficiency | |
|---|---|---|---|---|---|
| | | | | $WUE_{LDM}$ ($g.kg^{-1}$ water) | $WUE_{TDM}$ ($g.kg^{-1}$ water) |
| *I.amorphoides* | Control | 9.142a | 1.13c | 1.350a | 1.696 |
| | Moderate stress | 4.56b | 1.54b | 1.273ab | 1.645 |
| | Severe stress | 2.09c | 1.76a | 1.078b | 1.758 |
| | SE | 0.285 | 0.025 | 0.0601 | 0.0805 |
| *I.arrecta* | Control | 8.32a | 1.17c | 1.273 | 1.425 |
| | Moderate stress | 5.04b | 1.47b | 1.348 | 1.743 |
| | Severely stress | 2.09c | 1.75a | 1.244 | 1.792 |
| | SE | 0.464 | 0.0434 | 0.0770 | 0.169 |
| *I.coerulea* | Control | 7.89a | 1.07c | 1.170 | 1.159 |
| | Moderate stress | 4.02b | 1.50b | 1.163 | 1.154 |
| | Severe stress | 1.46c | 1.78a | 1.176 | 1.212 |
| | SE | 0.216 | 0.239 | 0.0374 | 0.0567 |
| *I.vicioides* | Control | 5.85a | 1.08c | 1.339b | 1.452b |
| | Moderate stress | 3.26b | 1.45b | 1.419ab | 1.632b |
| | Severe stress | 1.15c | 1.75a | 1.537a | 1.915a |
| | SE | 0.143 | 0.021 | 0.0311 | 0.0623 |

*Means with different letters between moisture levels within species differ at P <0.05.*

### 5.4.4. *Cumulative water use and water use efficiency*

For all species cumulative water use during the experimental period was significantly different between the three moisture levels. The water use efficiency responses of the species were, however, contrasting. Water use efficiency for leaf dry matter (fodder) production was significantly lower ($P < 0.05$) for *I. amorphoides*, and significantly higher for *I. vicioides*, at severe stress level, while the water use efficiency for leaf biomass production was not affected ($P > 0.05$) by moisture stress in the case of *I. arrecta* and *I. coerulea* (Table 5.5). The amounts of total dry matter produced per unit of water used were not significantly affected ($P > 0.05$) in *I. amorphoides*, *I. arrecta* and *I. coerulea*, while it was significantly increased ($P < 0.05$) by severe moisture stress in *I. vicioides* (Table 5.5).

### 5.4.5. *Transpiration and Transpiration efficiency*

The effect of moisture stress on cumulative transpiration, and transpiration efficiencies for leaf biomass production and total biomass production was variable amongst the different species (Table 5.5). The cumulative transpiration was significantly reduced ($P < 0.05$) in *I. amorphoides* and *I. coerulea* at the moderate stress level, while it was not affected in *I. arrecta* and *I. vicioides*. Transpiration efficiency, both in terms of leaf biomass or total biomass yield, tended to increase slightly in moderate stress treatments and then decline with an increasing level of moisture stress. The differences were not, however, statistically significant in *I. amorphoides*, *I. arrecta* and *I. coerulea* (Table 5.5).

**Table 5. 5.** Cumulative transpiration, water stress index based on transpiration and transpiration efficiencies based on leaf dry matter yield and total biomass of four *Indigofera* species grown under moisture stress and non-stress conditions**.**

| Species | Moisture level | Cumulative Transpiration ($kg.plant^{-1}$) | Water stress index based on T (1-T/PT) | Transpiration efficiency | |
|---|---|---|---|---|---|
| | | | | $TE_{LDM}$ ($g.kg^{-1}$ water) | $TE_{TDM}$ ($g.kg^{-1}$ water) |
| *I.amorphoides* | Control | 4.75a | 1.28b | 2.003 | 2.840 |
| | Moderate stress | 2.45b | 1.71a | 1.978 | 3.328 |
| | Severe stress | 2.53b | 1.69a | 1.398 | 2.390 |
| | SE | 0.282 | 0.0527 | 0.1556 | 0.2363 |
| *I.arrecta* | Control | 3.94 | 1.37b | 1.818 | 2.173 |
| | Moderate stress | 2.93 | 1.58ab | 2.080 | 3.098 |
| | Severely stress | 2.54 | 1.65a | 1.663 | 2.428 |
| | SE | 0.0464 | 0.0953 | 0.194 | 0.3478 |
| *I.coerulea* | Control | 3.51a | 1.19b | 1.648 | 1.618 |
| | Moderate stress | 1.90b | 1.68a | 1.660 | 1.665 |
| | Severe stress | 1.90b | 1.68a | 1.558 | 1.550 |
| | SE | 0.216 | 0.0661 | 0.109 | 0.1715 |
| *I.vicioides* | Control | 1.47 | 1.46 | 4.218ab | 6.045a |
| | Moderate stress | 1.15 | 1.74 | 6.413a | 10.588a |
| | Severe stress | 1.59 | 1.35 | 2.445b | 3.260b |
| | SE | 0.143 | 0.127 | 0.7104 | 1.296 |

Means with different letters between moisture levels within species differ at $P < 0.05$.

## 5.5. Discussion

Leaf production and the rate of leaf area expansion are critical to maximize canopy size and subsequently enhance the photosynthetic process and biomass accumulation (Monteith 1977; Squire 1990). In this study moisture stress had no effect on the canopy size (total leaf area) of *I. coerulea* and *I. arrecta*, but it significantly reduced total leaf area of *I. amorphoides* at the moderate stress level and that of *I. vicioides* at the severe moisture stress level. Different mechanisms were involved as a coping strategy to compensate for the adverse effect of stress on growth, development, dry matter production and survival of the plants. A reduction of total leaf area was evident in *I. amorphoides* as a result of moisture stress and this was mainly dueto its direct effect on leaf production, i.e. decreasing leaf number and leaf area expansion rate. In *I. vicioides*, however, the reduction in leaf area was mainly due to the low leaf area expansion rate. The reduction in leaf area is a typical characteristic of drought avoider plants (Quilambo 2000), which cope with moisture deficit stress through increasing water acquisition or conservation of water, which otherwise may have been lost through transpiration. The reduction in leaf production and leaf area expansion rate, in response to moisture stress, has been reported elsewhere (Norris 1982; De Costa 1997). Similarly, although plant responses to moisture stress decreases leaf size, considerable genetic variation is expected between plants of the same or different species (McCree and Davis 1974; Yae *et al.* 1988). In this study, however, leaf size, as measured in terms of mean area per leaf, was not affected by moisture stress in the *Indigofera* species under investigation.

The *Indigofera* species have exhibited variation in terms of their sensitivity to available water or moisture deficit stress, and this was reflected in terms of growth, dry matter yield and dry matter allocation response. Plant growth was not significantly affected in *I. arrecta*, *I. coerulea* or *I. vicioides* plants subjected to moisture deficit stress. Moisture deficit stress did, however, decrease relative growth rate in *I. amorphoides* and this was mainly due to the direct effect on decreasing leaf area ratio of the plants. Growth rate is affected by water deficit stress because of the following two situations: a reduction in leaf area expansion rate, which in turn is the result of loss of turgor; or a reduction in the rate of photosynthesis due to closing of the stomata (McCree 1974). Other growth parameters, such as specific leaf weight, specific leaf area and nett assimilation rate, were not affected by moisture stress in any of the species. It is well known that plants may develop plasticity in their leaf mass ratio to compensate for the limited plasticity in their leaf mass per area. In contrast to these findings, the production of leaves with smaller leaf area and a lower rate of photosynthesis were reported for faba bean grown under water deficit (Husain *et al.* 1990; Xia 1994). A decrease in specific leaf area or an increase in specific leaf weight (leaf mass per area) were reported for some crops (Ishizaki *et al.* 2003) and this may be mainly due to the accumulation of non-structural carbohydrates (Poorter *et al.* 1997), which benefit the plants by minimizing excessive transpiration loss and increasing the nett assimilation rate through maintaining high leaf nitrogen content per area. Since nitrogen is an essential constituent of proteins, a decrease in the plant N concentration may lead to a reduction in plant function and *vise versa*, particularly in photosynthetic capacity (Field and Mooney 1986; Luo *et al.* 1994).

As with relative growth rate, the total biomass yields of *I. arrecta*, *I. coerulea* and *I. vicioides* were not affected by moisture stress. Some of these species seemed to have an osmotic adjustment mechanism, since leaf area, normally a very sensitive parameter to drought, was not significantly affected by moisture deficit stress in either *I. arrecta* or *I. coerulea*. The adverse effects of moisture stress on some physiological and morphological processes, such as total leaf area, leaf senescence, relative growth rate, leaf area ratio and nett assimilation rate, were relatively minimal. Furthermore, drought stress tolerance indices for *I. arrecta* and *I. coerulea*, grown under no stress and moisture stressed conditions, were not different. In contrast, severe moisture deficit stress resulted in a reduced total biomass yield of *I. amorphoides*, and this was reflected in lower drought stress tolerance indices of the stressed treatments. The observed reduction in biomass yields agrees with findings of Husain *et al.* (1990) and Xia (1994) who reported a decrease in dry matter production of faba beans under water deficit conditions. The reduction in total biomass yield of *I. amorphoides* under water stress was as a result of reduction in leaf area, which is the main site of assimilate production. The stem and root yields were the most affected, and significantly reduced the total biomass in *I. amorphoides.* Whereas only leaf yields were reduced in *I. vicioides*, as a result of severe moisture stress, this was probably due to preferential allocation of more assimilate to the root mass fraction and/or a decrease in rate of photosynthesis per unit leaf dry mass, which in turn is the result of either a decrease in water uptake per unit root mass or a reduced nutrient uptake by plants that grow under limited water supply (Poorter and Nagel 2000).

The *Indigofera* species evaluated also exhibited variation in terms of dry matter allocation responses. Variation in the leaf mass fractions was not observed between stressed and non-stressed plants of any of the species. However, the root mass fractions of *I. amorphoides* and *I. arrecta* were substantially increased in plants subjected to severe moisture stress. This agrees with Husain *et al.* (1990), who reported an increased root dry matter yield for faba beans subjected to water stress. Under field conditions the implication is, that the biomass allocation to roots increases with a decreasing water availability as this would enable better exploitation of soil water reserves and may, thus, confer increased drought resistance to the species (Turner and Begg 1981). These shifts in allocation of assimilates could be seen as an adaptive mechanism, enabling the plants to capture more of the resources that most strongly limit plant growth.

Water use efficiency is an important physiological characteristic, which is directly related to the ability of the plants to cope with water deficit stress. WUE on an annual basis exhibits a ten-fold range from 0.002 to 0.02 g dry matter per g of water, irrespective of photosynthetic biochemistry ($C_4$ vs $C_3$), in semi-arid grasslands (Pearson and Ison 1997). Some grassland species may use more water per unit of dry matter accumulated than others, and most species may have the same relative sensitivity to available water, but the species, which use less water per unit dry matter increment have the highest water use efficiency, which confers ecological advantage. In this experiment higher water use efficiencies were exhibited by *I. vicioides* under severe stress condition than by non-stressed plants. This is consistent with the findings of Craufurd *et al.* (1999), who reported increased water use efficiency of potted peanut plants under water stress (50%

versus 100% maximum available soil water). Transpiration efficiency was, however, higher at moderate moisture deficit stress than in non-stressed or severely stressed plants. This species maintained a high relative growth rate and this parameter was relatively less sensitive to drought. According to Sinclair *et al*. (1984), stomatal control (closure during midday periods), acting to prevent high transpiration rates, could significantly improve water use efficiency. Specific leaf area has been shown to be well inversely correlated with WUE (Wright *et al*. 1994) and TE (Turner *et al.* 2001). Leaf ash content, and its elements, expressed on dry-matter basis have been shown to be significantly correlated with TE in a number of species (Masle *et al*. 1992; Mayland *et al.* 1993; White 1997).

Generally the *Indigofera* species have shown variation in terms of their response to a range of moisture levels. The results obtained in the present study suggest that *I. amorphoides* was relatively more sensitive to moisture deficit stress than the other species, while *I viciodes* was more tolerant, considering the negative effect of moisture deficit stress on leaf area, growth, dry matter accumulation and water use efficiency. The sensitivity of a growth parameter, or plant part, seemed to depend on the inherent strategy of the species in question. The effect of moisture deficit stress on growth parameters, in particular, was small compared to its effects on leaf area and biomass allocation pattern. This was not surprising taking into consideration the adaptability of the species and collection environment of these particular accession, where water stress is a common phenomena. Further research should contribute to the determination of the exact mechanisms, which allows *I. vicioides* to maintain its growth unabated under water stress.

# CHAPTER 6

# The influence of season and species on forage quality of five *Indigofera* accessions

## 6.1. Abstract

Two experiments were undertaken to determine the influence of season/year and species on forage quality of *Indigofera* accessions. In Experiment 1, Leaf material was collected from five *Indigofera* species (*I. amorphoides*, *I.arrecta*, *I. brevicalyx*, *I. costata* and *I. cryptantha*) by harvesting plants in the autumn of 2003 and the autumn of 2004. In Experiment 2, edible forage (leaves+ <3mm stem) material collected by harvesting plants in the autumn of 2004 and the spring of 2004. Both leaf meal and edible forage material were analyzed for dry matter (DM), ash, crude protein (CP), neutral detergent fibre (NDF), *in vitro* digestible organic matter content (IVDOM), and Ca, P, Mg, Cu, Zn and Mn concentrations. In Exp. 1, the interaction effect between species and year of cutting was significant ($P< 0.05$) in terms of all studied parameters, except Cu concentration. In Exp. 2, significant interactions ($P< 0.05$) were revealed between species and season effect for ash content as well as Ca and Mg concentrations in the edible material. The CP content of the leaf meal ranged between 223 to 311 g/kg DM and that of edible forage material ranged between 81 to 287 g/kg DM. Spring growth had significantly higher ($P< 0.05$) CP content than autumn growth in all species. The NDF content of leaves ranged between 189 g/kg DM in *I. amorphoides* to 504 g/kg DM in *I. costata*, while that in edible material ranged between 328 g/kg DM in *I. arrecta* to 654 g/kg DM in *I. cryptantha*. The *in vitro* digestibility of leaves ranged between 568 g/kg DM in *I. cryptantha* to 717 g/kg DM in *I amorphoides*, while that of edible material ranged between 507 to 722 g/kg DM in *I. cryptantha*. In contrast to the trend with CP and NDF, *in vitro* digestibility of dry material tended to decrease from the spring of 2004 to the autumn of 2004 harvest. Higher levels of Ca, P, Mg, Zn and Cu concentration were revealed in the leaf meal of the first harvest than in the re-growth harvest. All of the species had Ca, Mg, Zn and Mn concentration levels that could support the requirements of ruminants. P and Cu were slightly deficient for some of the species in the autumn harvest. It is, therefore, essential to supplement P and Cu from other sources during this period to meet the requirement of the animals.

**Key words**: crude protein, forage, *Indigofera*, *in vitro* digestibility, mineral composition and neutral detergent fibre

## 6.2. Introduction

Forage quality is usually determined by animal performance when forages are fed to livestock. The main determinants of forage quality are nutrient concentrations (crude protein content, crude fibre content, etc.), intake, digestibility and partitioning of metabolised products within animals (Juiler *et al.* 2001). Most of these attributes are shown to be strongly affected by plant species, plant morphological fraction, environmental factors, and stage of maturity (Lambert *et al.* 1989; Papachristou and Papanastasis 1994).

Livestock managers in semi-arid areas are strongly challenged by the large temporal variability in climatic factors, which, in turn, affects forage production and quality. Maturity influences forage quality more than any other single factor, but plant environment and agronomic factors modify the impact of maturity on forage quality and cause year to year, seasonal, and geographical location effects on forage quality even when harvested at the same stage of development (Buxton 1996). Temperature is among the environmental factors that have a direct influence on forage quality (Wilson 1977). A rise in temperature increases cell wall constituents, increases lignification, decreases soluble carbohydrate concentration and decreases digestibility (Pearson and Ison 1997). It also reduces the leaf: stem ratio of the forage, which directly affects the digestibility of the forage dry matter because of the lower digestibility of the stems in relation to the leaf (Buxton *et al.* 1995). The digestibility of forages decreases by about 0.5 to 7 percentage units per 1°C increase in temperature. This means that forages grown in cooler regions, or seasons, are of higher quality than forages grown in warmer climates. Similarly, the

concentration of mineral elements in forages is dependent upon the interaction of a number of factors, including soil, plant species, stage of maturity, yield, pasture management and climate (McDowell 2003).

The objectives of the present study were to investigate the effect of species on the quality of leaves and edible material of five *Indigofera* species, and determine the effects of season/year on chemical composition and *in vitro* digestibility of the different species.

## 6.3. Materials and Methods

### *6.3.1. Site and experimental field management*

The study was conducted at the University of Pretoria, Hatfield Experimental Farm. Seeds of *Indigofera* accessions were sown in mid November 2002 in trays in a nursery. After establishment, a total of 54 seedlings of each accession were transplanted into field plots in January 2003 for a characterization study (Hassen et al. 2005). Eighteen seedlings were planted in 1.5 m x 3 m plots with a spacing of 50 cm between rows and plants. Each accession was replicated 3 times. Spacings of 50 and 100 cm were maintained between adjacent plots and blocks, respectively. The plants were irrigated twice per week for 2 hours depending on rainfall events. Plots were kept weed-free by hand pulling. Two experiments were carried out by sampling the leaves, or edible forage, of five *Indigofera* species (i.e., *I. amorphoides 7570*, *I. arrecta 10479*, *I. brevicalyx 7848*, *I. costata 8712* and *I. cryptantha 7070*) from the same plants that were harvested according to schedules indicated in Exps. 1 and 2.

### *6.3.2. Experiment 1. Effect of year and species on leaf meal quality*

In this experiment, samples of leaf material, hereafter termed 'leafmeal', from the five *Indigofera* species were collected in the autumn of 2003 and again in the autumn of 2004. All the plots were clear-cut before the commencement of the 2003/2004 season allowed to grow until harvested in the autumn of 2004. Above ground biomass was harvested from a total of 6 plants and separated into leaf and stem components before being dried in forced-draught oven at 70 ºC for 48 hours for subsequent laboratory analyses. Dried leaf material was milled to pass through a 1mm sieve and kept in an airtight container for later laboratory analyses. The leaf fractions of each plot, for all the species, were analysed separately for the determination of percentage dry matter (DM), and ash, according to AOAC (2000) procedure 942.05 and total nitrogen (N), according to AOAC (2000) procedure 968.06. The neutral detergent fibre (NDF) was determined according to the procedure of Robertson and Van Soest (1981). The NDF was assayed without the use of heat stable amaylase and expressed inclusive of residual ash. *In vitro* digestibility of organic matter (IVDOM) was determined following the procedure of Tilley and Terry (1965), as modified by Engels and Van der Merwe (1967). The mineral composition of the forage was determined according to AOAC (2000) under procedure 965.17 for phosphorous (P) and under procedures 935.13 A(a) for the other minerals including calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn) and manganese (Mn) concentrations.

### *6.3.3. Experiment 2. Effect of season and species on the quality of edible Indigofera forage*

In this experiment, samples of leaves plus fine stem fractions (<3mm in stem diameter), hereafter termed as 'edible forage' material, were collected from re-growth of all 5 species in Exp. 1 in the autumn of 2004 and the spring of 2004 to assess variation in the nutrient quality of edible forage between a seasons and species. The total biomass of 6 plants from the middle row of each plot was harvested. This was separated into leaf, fine stem (< 3mm diameter) and coarse stem (>3mm stem diameter) fractions. The edible forage samples were prepared by mixing the leaves and fine stem fractions. This was dried and subsequently milled to pass through a 1mm sieve and kept in airtight containers for later laboratory analyses. The edible forage portions of each plot, for all species, were analysed separately for DM, ash, N, NDF, IVDOM content, and the Ca, P, Mg, Cu, Zn and Mn concentrations. The same procedures were used as in Exp. 1.

### *6.3.4. Statistical analyses*

All parameters measured in Exps. 1 and 2 were analyzed using Proc GLM of SAS (2001). The model included effects of species, year/season and the interaction. Where F ratio has shown significance for either of the main or interaction effects, difference between least squares means were tested using PDIFF option of SAS (2001), which computes probabilities for all pair wise differences. Interactive means were used along their common standard error of the means for the tabular presentation.

## 6.4. Results

### *6.4.1. Experiment 1. Effect of year and species on leaf meal quality*

There were interaction effects between species and year of harvest (P< 0.05) with respect to ash, CP, NDF, IVDOM, Ca, P, Mg, Zn and Mn. The exception was Cu (Tables 1 and 2).

In autumn of 2003 the CP content of *I. cryptantha* was higher (P< 0.05) than that of *I. costata*, *I. brevicalyx* and *I. arrecta*. In contrast, the CP contents of *I. costata* and *I. brevicalyx* in the autumn of 2004 were higher (P< 0.05) than the other species (Table 1). In the first harvest, the NDF content of *I. brevicalyx* and *I. arrecta* was higher than that of *I. amorphoides* while in the second harvest, the NDF content of *I. costata* was higher than *I. brevicalyx* and *I. amorphoides*. The IVDOM of *I. costata* and *I. brevicalyx* in the autumn of 2003 were lower (P< 0.05) than that of the other species. In contrast, the IVDOM of *I. brevicalyx* in the autumn of 2004 was higher (P< 0.05) than that of *I. amorphoides*, *I. cryptantha* and *I. costata* (Table 1).

Among the species, *I. cryptantha* had the lowest Ca concentration in the autumn of 2003 while *I. arrecta* had lower Ca concentration, compared to the other species, in the harvest of 2004 (Table 2). *I. costata* had a lower P concentration compared to *I. cryptantha* in 2003 while *I. cryptantha* and *I. arrecta* had the lowest P concentration compared to other species in 2004. *I. amorphoides* had a higher Mg concentration than the other species in the first harvest, whereas in the re-growth harvest the concentration of Mg in *I. brevicalyx* was the highest compared to the other species. Generally the Cu concentration was not

**Table 6. 1.** Chemical composition and *in vitro* digestibility of the leaf meal of five *Indigofera* species, as influenced by year and species.

| Year | Species | Ash (g/kg DM) | CP (g/kg DM) | NDF (g/kg DM) | IVDOM (g/kg DM) |
|---|---|---|---|---|---|
| 2003 | *I. amorphoides* | 133.7 Aa[1] | 266 Aab | 189 Bb | 717 Aa |
| | *I. cryptantha* | 90.7 Ab | 297 Aa | 222 Bab | 707 Aa |
| | *I. costata* | 133.7 Aa | 226 Bb | 225 Bab | 655 Ab |
| | *I. brevicalyx* | 96.4 Ab | 255 Bbc | 255 Ba | 655 Ab |
| | *I. arrecta* | 121.9 Aa | 253 Abc | 242 Ba | 702 Aa |
| 2004 | *I. amorphoides* | 57.9 Bc | 223 Be | 402 Ae | 598 Bde |
| | *I. cryptantha* | 55.2 Bc | 244 Be | 457 Acd | 568 Be |
| | *I. costata* | 49.6 Bc | 311 Ad | 504 Ac | 558 Be |
| | *I. brevicalyx* | 70.4 Bc | 291 Ad | 422 Ade | 666 Ac |
| | *I. arrecta* | 59.1 Bc | 246 Ae | 465 Acd | 631Bcd |
| SEM | | 7.67 | 11.7 | 16.6 | 15.3 |
| Significance level (P) | | | | | |
| Species | | 0.0610 | 0.0767 | 0.010 | 0.0062 |
| Year | | 0.0001 | 0.6185 | 0.0001 | 0.00012 |
| Species x Year | | 0.0049 | 0.0001 | 0.0273 | 0.0012 |

[1]For each studied parameter, column means within the same year followed by the same lower case letter or column mean within the same species followed by the same upper case letter are not significantly different (P>0.05).

**Table 6. 2.** Mineral composition of leaves of *Indigofera* species, as influenced by species and year.

| Year | Species | Ca (g/kg) | P (g/kg) | Mg (g/kg) | Cu (mg/kg) | Zn (mg/kg) | Mn (mg/kg) |
|---|---|---|---|---|---|---|---|
| 2003 | *I. amorphoides* | 38.7 Aab | 2.6 Abc | 10.7 Aa | 11.8 | 48.4 Aa | 148.0 Bb |
| | *I. cryptantha* | 26.6 Ac | 3.3 Aa | 3.9 Ad | 10.9 | 50.2 Aa | 137.4 Bb |
| | *I. costata* | 45.2 Aa | 2.3 Ab | 4.6 Acd | 13.3 | 35.0 Aa | 153.1 Bb |
| | *I. brevicalyx* | 32.2 Abc | 3.0 Aab | 5.2 Bc | 15.3 | 47.4 Aa | 142.5 Bb |
| | *I. arrecta* | 37.9 Aab | 2.8 Aabc | 6.5 Ab | 13.7 | 45.4 Aa | 186.0 Ba |
| 2004 | *I. amorphoides* | 17.9 Bd | 2.6 Ad | 4.4 Bf | 8.8 | 30.3 Bc | 281.3 Ac |
| | *I. cryptantha* | 13.4 Bde | 1.9 Be | 3.2 Ag | 10.8 | 50.9 Ab | 279.8 Ac |
| | *I. costata* | 12.2 Bde | 2.5 Ad | 4.1 Agh | 9.5 | 48.6 Ab | 210.6 Ad |
| | *I. brevicalyx* | 14.4 Bde | 2.8 Ad | 6.5 Ae | 10.2 | 39.4 Abc | 213.2 Ad |
| | *I. arrecta* | 9.7 Be | 1.9 Be | 2.1 Bi | 9.0 | 27.2 Bc | 227.3 Ad |
| SEM | | 3.06 | 0.18 | 0.33 | 1.21 | 5.46 | 7.13 |
| Significance level (P<) | | | | | | | |
| Species | | 0.0530 | 0.0395 | 0.0001 | 0.4126 | 0.1491 | 0.0001 |
| Year | | 0.0001 | 0.0013 | 0.0001 | 0.0003 | 0.0979 | 0.0001 |
| Species x Year | | 0.0308 | 0.0016 | 0.0001 | 0.2797 | 0.0440 | 0.0001 |

[1]For each studied parameter, column means within the same year followed by the same lower case letter or column mean within the same species followed by the same upper case letter are not significantly different (P>0.05).

significantly different (P> 0.05) between species. Differences in the Zn concentrations among the *Indigofera* species were significant (P< 0.05) only in 2004. *I.cryptantha* and *I. costata* had the highest Zn concentrations, which were significantly higher (P< 0.05) than *I. amorphoides* and *I. arrecta*. *I. arrecta* had the highest Mn concentration in the first harvest while *I. amorphoides* and *I. cryptantha* had higher (P< 0.05) Mn concentrations in the second harvest.

*I. amorphoides* and *I. cryptantha* had higher (P< 0.05) CP contents in the autumn of 2003 than in the autumn of 2004, while *I. costata* and *I. brevicalyx* had a higher (P< 0.05) CP content in 2004 than in 2003 (Table 1). In all species the NDF content in the autumn of 2004 was higher (P< 0.05) than in 2003 and *In vitro* digestibility of all the species, except *I. brevicalyx*, was significantly higher in 2003 than in 2004. All *Indigofera* species had lower (P< 0.05) Ca concentrations in 2004 than in 2003 (Table 2). Both *I. cryptantha* and *I. arrecta* had significantly lower (P< 0.05) P concentrations in 2004 than in 2003. *I. amorphoides* and *I. arrecta* had lower (P< 0.05) Mg concentrations in 2004 than in 2003, whereas *I. brevicalyx* had a significantly higher Mg concentration in 2004 than in 2003. The copper content in 2003 is generally higher (P< 0.05) than in 2004. *I. amorphoides* and *I. arrecta* had lower (P< 0.05) Zn concentrations in 2004 than in 2003. In general, Mn concentrations were higher in 2004 than in 2003 for all species.

### *6.4.2. Experiment 2. Effect of season and species on the quality of edible Indigofera forage*

There were interaction effects between species and season of harvest ($P < 0.05$) in ash content as well as the Ca and Mg concentrations of the edible forage (leaves and stem fraction with <3mm diameter) material (Table 3 and 4). In contrast the interaction effect between species and season of harvest was not significant ($P > 0.05$) in terms of CP, NDF, IVDOM, P, Cu, Zn and Mn concentration of the edible forage (Table 3 and 4).

Species differences in terms of ash content were not detected ($P > 0.05$) in the autumn harvest, while in the spring harvest *I. cryptantha* had a significantly higher ($P < 0.05$) ash content than *I. costata*, *I. amorphoides* or *I. brevicalyx* (Table 3). Generally the *Indigofera* species did not differ ($P > 0.05$) in terms of CP content, NDF content and *in vitro* digestibility (Table 3). The differences between *Indigofera* species, in terms of Ca and P concentrations, were generally not significant ($P > 0.05$). Among the species, however, *I. cryptantha* had a higher Mg concentration, compared to *I. brevicalyx*, *I. arrecta* and *I. amorphoides* in spring, while in autumn the Mg concentration of *I. amorphoides* was the highest compared to other species. The differences between the *Indigofera* species in terms of some micro-minerals such as Cu, Zn and Mn were, however, insignificant ($P > 0.05$).

All species had higher ($P < 0.05$) ash, CP and IVDOM in the spring growth than in the autumn growth. In contrast, for all species the NDF content of spring growth was lower ($P < 0.05$) than in autumn growth (Table 3). All *Indigofera* species, except *I. brevicalyx*,

**Table 6. 3.** Chemical composition and *in vitro* digestibility of edible forage (leaves plus <3mm stem) material of *Indigofera* species, as affected by species and season of growth.

| Season | Species | Ash (g/kg DM) | CP (g/kg DM) | NDF (g/kg DM) | IVDOM (g/kg DM) |
|---|---|---|---|---|---|
| Autumn | *I. amorphoides* | 51.2 Ba | 137 | 625 | 568 |
| | *I. cryptantha* | 45.0 Ba | 81 | 654 | 507 |
| | *I. costata* | 40.5 Ba | 127 | 622 | 521 |
| | *I. brevicalyx* | 45.0 Ba | 129 | 607 | 525 |
| | *I. arrecta* | 45.0 Ba | 182 | 595 | 535 |
| Spring | *I. amorphoides* | 60.6 Ad | 228 | 330 | 632 |
| | *I. cryptantha* | 82.2 Ab | 287 | 351 | 722 |
| | *I. costata* | 67.7 Acd | 262 | 347 | 677 |
| | *I. brevicalyx* | 62.1 Ad | 236 | 365 | 671 |
| | *I. arrecta* | 74.7 Abc | 261 | 328 | 655 |
| SEM | | 3.69 | 27.0 | 26.5 | 30.9 |
| Significance level (P) | | | | | |
| Species | | 0.0576 | 0.5533 | 0.6565 | 0.9713 |
| Season | | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| Species x Season | | 0.0313 | 0.1772 | 0.8035 | 0.2197 |

[1]For each studied parameter, column means within the same season followed by the same lower case letter or column mean within the same species followed by the same upper case letter are not significantly different (P>0.05).

**Table 6. 4.** Mineral composition of edible forage (leaves plus <3mm stem) materials of *Indigofera* species as affected by species and season of growth.

| Season | Species | Ca (g/kg) | P (g/kg) | Mg (g/kg) | Cu (mg/kg) | Zn (mg/kg) | Mn (mg/kg) |
|---|---|---|---|---|---|---|---|
| Autumn | *I. amorphoides* | 10.3 Ba | 1.1 | 5.0 Aa | 9.07 | 31.1 | 143.8 |
| | *I. cryptantha* | 12.0 Ba | 1.0 | 2.1 Bb | 9.13 | 51.8 | 139.3 |
| | *I. costata* | 9.9 Ba | 1.0 | 1.9 Bb | 10.19 | 27.1 | 164.9 |
| | *I. brevicalyx* | 13.8 Aa | 1.3 | 2.9 Bb | 9.23 | 49.2 | 117.1 |
| | *I. arrecta* | 12.0 Ba | 1.5 | 2.4 Bb | 11.0 | 41.8 | 165.4 |
| Spring | *I. amorphoides* | 21.2 Ab | 2.4 | 4.5 Ad | 10.4 | 51.8 | 125.8 |
| | *I. cryptantha* | 18.2 Ab | 2.9 | 6.1 Ac | 10.1 | 53.1 | 169.6 |
| | *I. costata* | 17.3 Ab | 2.7 | 4.8 Acd | 11.1 | 51.4 | 214.8 |
| | *I. brevicalyx* | 16.1 Ab | 2.1 | 4.7 Ad | 11.8 | 42.2 | 218.9 |
| | *I. arrecta* | 19.6 Ab | 2.3 | 4.6 Ad | 9.62 | 47.4 | 345.7 |
| SEM | | 1.22 | 0.25 | 0.48 | 0.95 | 6.16 | 38.89 |
| Significance level (P<) | | | | | | | |
| Species | | 0.3954 | 0.7859 | 0.0795 | 0.7352 | 0.2962 | 0.0539 |
| Season | | 0.0001 | 0.0001 | 0.0001 | 0.1744 | 0.0335 | 0.0119 |
| Species x Season | | 0.0371 | 0.1330 | 0.0030 | 0.3765 | 0.0999 | 0.1558 |

[1]For each studied parameter, column means within the same season followed by the same lower case letter or column mean within the same species followed by the same upper case letter are not significantly different (P>0.05).

had higher (P< 0.05) Ca concentrations in the spring than in the autumn. All *Indigofera* species had higher P, Zn amd Mn concentrations in spring than in autumn. All *Indigofera* species, except *I. amorphoides*, had significantly higher Mg concentration in spring growth than in autumn growth. Season of growth had no significant effect on the Cu content of the edible forage.

**6.5. Discussion**

The nutritive value of forages depends upon a number of factors including plant species or varieties, growing conditions (soil, climate, grazing, etc.), plant fraction and the stage of maturity at sampling (Wilson 1977; Lambert *et al.* 1989; Papachristou and Papanastasis 1994; Buxton *et al.* 1995; Pearson and Ison 1997). Maturity influences forage nutritive value more than any other single factor, but environmental and agronomic factors may modify the impact of maturity and cause variation between years, seasons, and geographical locations, even when harvested at the same stage of development (Buxton 1996). Temperature is the major environmental factor that may have a direct influence on maturity and consequently on forage quality (Wilson 1977). Generally forages grown in cooler regions, or seasons, are of higher quality than forages grown in warmer environments (Pearson and Ison 1997). Plant growth is relatively sluggish in winter due to the negative effect of low temperature on growth. In spring, however, growth is most active with a peak in summer when temperatures are high and this cause fast growth and maturity of plants. This means that from a nutritional point of view high temperature will increase cell wall constituents and lignification, while decreasing the CP, soluble carbohydrate concentration and digestibility of the forage

(Buxton *et al.* 1995; Pearson and Ison 1997). This was supported by the results of this study, which revealed higher CP and lower NDF contents of the edible forage in spring than autumn for all the species. In this study, the IVDOM of the forage was higher in the spring of 2004 than the autumn of 2004. A similar pattern of high CP (Papachristou and Papanastasis 1994; Ammar *et al.* 2004), low NDF (Shayo and Udén 1999; Ammar *et al.* 2004) and high digestibility (Ammar *et al.* 2004) values has been reported in spring growth than in autumn for other browse species. In this study, however, the lowest CP content recorded for *I. cryptantha* (81 g/kg DM) in the autumn of 2004 is still slightly more than the minimum threshold level (80 g CP/kg DM), which would limit intake of tropical forages (Minson 1980). Pearson and Ison (1997) also indicated that the digestibility of forages decreases by about 0.5 to 7 percentage units per 1°C increase in temperature.

On the other hand, the effects of year on the nutritive value of *Indigofera* leaf meal were not conclusive. Contrary to low CP, high NDF and low IVDOM content of forage as a result of increase in environmental temperature (Buxton *et al.* 1995; Pearson and Ison 1997), high CP, low NDF and high IVDOM content were observed in this study for *I. amorphoides* and *I. cryptantha* harvested in a relatively warm year (the autumn of 2003) than cool year (autumn 2004). The reason for this is not clear, probably it is due to the confounding effect of some other factors (e.g. length of the re-growth period) might have interacted with temperature to ultimately modifies the influence of temperature on plant maturity and subsequently forage quality.

Forage from leguminous shrub and tree species is known for its high protein content through out the year due to the ability of these plants to fix atmospheric nitrogen (Tolera *et al.* 1997; Hove *et al.* 2001; Ammar *et al.* 2004). In this study, the CP content of the leaf meal of the five *Indigofera* species ranged between 223 to 311 g $kg^{-1}$ DM, while that of edible forage ranged between 81 to 287 g/kg DM. In terms of the CP content alone, most of the species can, therefore, regarded as medium to high quality forages. The maximum cell-wall concentration (NDF) of diets, that will not hinder intake and animal production, can be as high as 700-750 g NDF/kg DM for mature beef cows and as low as 150-200 g NDF/kg DM for finishing ruminants. The optimum concentration of NDF in diets of high-producing dairy cows, at peak lactation, is 270-290 g/kg DM, which allows for adequate energy and maintains adequate fibre in the diet (Mertens 1994). In the present study, the NDF content of the leaf meal ranged between 189 g/kg DM in *I. amorphoides* to 504 g/kg DM in *I. costata, while* the NDF content of the edible forage ranged between 328 g/kg DM in *I. arrecta* to 654 g/kg DM in *I. cryptantha.* The *in vitro* digestible organic matter content of the leaf meal ranged between 568 g/kg DM in *I. cryptantha* to 717 g/kg DM in *I. amorphoides*. Both low and high *in vitro* digestibility values for edible forage were recorded in *I. cryptantha* in autumn (507 g/kg DM) and in spring (722 g/kg DM) harvest, respectively. Differences in digestibility are primarily associated with the chemical composition of the samples, especially with their cell wall and CP contents. The cell wall fractions may negatively affect browse digestibility (Wilson, 1977). According to Van Soest (1994), cell contents are readily and completely digested, whereas cell walls are slowly and only digested to a certain extent, depending on the degree of lignifications. The concentration of individual minerals in forages varies greatly depending on soil,

plant, and management factors (Greene *et al.* 1987; Haenlein 1980; 1991). A peak generally occurs during spring when growth is most active and levels decline steadily reaching the lowest levels during winter (Huston *et al.* 1981). This was supported by the results of this study, which revealed higher levels of Ca, P, Mg, Zn and Mn concentration in the forage in spring than in autumn. In the present study, levels of Ca, P, Mg, Cu and Zn concentration were also shown to be higher in the leaf meal of first harvest than of re-growth harvest. According to McDowell (2003), the critical levels of mineral concentration for ruminant requirements are 3 g/kg DM for Ca, 2.0 g/kg DM for Mg, 10 mg/kg DM for Cu, 30 mg/kg DM for Zn and 30-40 mg/kg DM for Mn. Despite the year-to-year and/or seasonal variation, the leaf meal or edible forage of all of *Indigofera* species, included in this study, had Ca, Mg, Zn and Mn concentration levels that could support the requirement of ruminants (Table 2 and 4). The levels of P concentration in autumn in all *Indigofera* species, however, ranged between 1-1.5 g/kg DM, which is far lower than 2.5 g P/kg DM, which is the critical level recommended to meet ruminants requirements. Copper was also slightly deficient in autumn edible forage of *I. amorphoides*, *I. cryptantha* and *I. brevicalyx*, and leaf meal harvested in the autumn of 2004, of *I. amorphoides*, *I. costata* and *I arrecta*. This study has demonstrated that the CP contents of the *Indigofera* accessions were sufficiently high to consider these species as potential protein supplements to low quality diets. In comparison with reports on the digestibility of conventional forages and browse, the present results indicate that forage from these five *Indigofera* species can be considered as highly digestible. Cell wall contents tended to increase whereas Ca, P, Mg, Zn, Mn, CP, and digestibility showed a tendency to decline from the highest values in spring to lower values in autumn with

advancing maturity. These results also indicate that the *Indigofera* species could safely meet the Ca, Mg, Zn and Mn requirement of ruminant animals, while it is essential to supplement P and Cu from other sources to meet the ruminant requirements.

# CHAPTER 7

# Intake and *in vivo* digestibility of *Indigofera* forage, compared to *Medicago sativa* and *Leucaena leucocephala* forage, by Merino sheep

## 7.1. Abstract

The voluntary intake and *in vivo* digestibility of forage from three different species (*Indigofera*, *Medicago sativa* and *Leucaena leucocephala*) were determined using five Merino sheep per experimental diet. Both *M. sativa* (lucerne) and *Leucaena* forage had higher (P< 0.05) crude protein (CP) and lower neutral detergent fibre (NDF) content than *Indigofera* forage. However, the apparent dry matter digestibility (DMD%) and organic matter digestibility (OMD%) coefficients for *Leucaena* forage were significantly lower (P< 0.05) than either *Indigofera* or *M. sativa* forage. The forage species had differed significantly (P< 0.05) in terms of apparent CP digestibility (CPD%) and NDF digestibility (NDFD%). *Indigofera* forage had a higher CPD% and NDFD% than *Leucaena* forage. The forage species also had significant differences (P< 0.05) in terms of dry matter intake in g $head^{-1}$ $day^{-1}$ (DMI), organic matter intake (OMI) and crude protein intake (CPI), but not in terms of neutral detergent fibre intake in g $head^{-1}$ $day^{-1}$ (NDFI). The difference between *Indigofera* and *Leucaena* forage in terms of DM intake per unit of metabolic body weight (DMI g $BW^{-0.75}$ $day^{-1}$) was not significant (P> 0.05), but forage DMI (g $BW^{-0.75}$ $day^{-1}$) of both *Indigofera* and *Leucaena* were significantly lower (P< 0.05) than forage DMI (g $BW^{-0.75}$ $day^{-1}$) of *M. sativa*. Merino sheep on *Indigofera* forage had the lowest CPI (g $BW^{-0.75}$ $day^{-1}$) as compared to CPI (g $BW^{-0.75}$ $day^{-1}$) of *M. sativa* and *Leucaena* forage. However, the digestible organic matter intake (DOMI) and digestible crude protein intake (DCPI) of Merino sheep on *Indigofera* forage was similar to that of sheep fed on *Leucaena*. In this study, lack of differences between *Indigofera* and *Leucaena* forage in terms of DOMI, DCPI and DNDFI means that *Indigofera* forage would likely support similar weight gains as that of *Leucaena*, but lower than that of *M. sativa* forage.

**Keywords**: Forage, *Indigofera*, intake, *in vivo* digestibility, *Leucaena*, Lucerne, organic matter intake.

## 7.2. Introduction

Chemical composition and digestibility value of feeds often provides information on the potential quality of the feed. However, the prediction of animal performance requires an accurate estimation of intake by the target animal. Thus, the quantity of dry matter voluntarily eaten by an animal is the most important factor, in determining the feeding value of a feed. Intake determines the amount of nutrients available for production above that required for maintenance (McDonald *et al.* 2002). It is more closely related to the rate of digestion of diets than the digestibility *per se*, although the two are generally related to one another. In ruminant animals, intake is limited by the rate of digestion of digestible material and the rate of passage of undigested material. These two determine the extent of digestion. It is to be expected that feeds with a low intake are not able to support high levels of animal production no matter how high the protein or mineral content of each unit of feed (Milford and Minson 1968). Therefore, intake is more important than digestibility in affecting production. Thus far, only limited information has been documented with respect to the intake of *Indigofera* species forage and those have been restricted to rat bioassay studies (Strickland *et al.* 1987).

The present study was undertaken to assess voluntary intake and *in vivo* digestibility of *Indigofera* forage, as compared to *Medicago sativa* and *Leucaena leucocephala*, by Merino sheep.

## 7.3. Materials and Methods

### *7.3.1. Site and experimental diet*

The study was conducted on the Hatfield Experimental Farm, University of Pretoria. The *Indigofera* forage was collected as a bulk harvest from a characterization experimental area. The details of field layout, spacing and management practices were provided by Hassen *et al*. (2005). Because of difficulties in producing adequate quantities of edible forage from a single species, the bulk forage material was collected from the different plots and/or various accessions, of mainly *I. arrecta*, *I. amorphoides*, *I. cryptantha*, and *I. brevicalyx* in order of their importance. These were mixed thoroughly before being used in the intake study. However, enough forage from single varieties of *M. sativa* and *L. leucocephla* were produced. The total biomass for *Indigofera* and *Leucaena* was separated into leaf, fine stem (< 3mm diameter) and coarse stem (>3mm stem diameter) portion. Subsequently the leaves and fine stem fractions were mixed to form the edible forage material, which was used in this study.

### *7.3.2. Experimental procedure and animal management*

In this experiment the intake and *in vivo* digestibility of edible *Indigofera* forage (leaves and <3mm stem fractions) was evaluated along with *Medicago sativa* and *Leucaena leucocephala*, using five Merino sheep per experimental diet maintained separately in metabolic cages. The animals were used in the experiment after the approval of the ethics committee of the University of Pretoria (project number AUCC050408008). A total of 15 Merino sheep (mean live weight of 62.6±13.44 kg) were provided with the three forages being studied. The sheep had free access only to the test forages and water throughout the

experimental period. During the trial, each animal had access to only one of the three experimental diets as unique feed. The intake and digestibility trial consisted of 10 days of adaptation followed by 7 days of data collection. Each animal was weighed to 0.1kg on the final day of the experimental period. Daily feed intake and faecal production was also measured for each animal. Total daily faecal production for each animal was stored frozen, at –10 ºC, until after completion of the collection period. The bulked faecal output from each animal and forage species were then weighed, thoroughly mixed and 10% of the weight sub-sampled prior to subsequent laboratory analyses. One sample of the forage dry matter on offer was taken every day, dried in a forced air oven at 60 ºC to constant weight and then ground through a l mm screen in a mill. These samples were analysed for the determination of DM, OM, CP, NDF content and IVDOM% and Indospicine concentration. The DM, ash and Nitrogen concentrations of each sample were determined following standard procedure (AOAC 2000). Crude protein was determined from N concentration by multiplying with 6.25. Mineral content was determined following AOAC procedures (2000). *In vitro* organic matter digestibility (IVDOM) was determined using the Tilley and Terry (1963) procedure, as modified by Engels and Van der Merwe (1967).

### *7.3.4. Statistical analysis*

All studied parameters were subjected to analysis of variance using proc GLM of SAS (2001). The model included the effect of forage species and where F ratios showed significance, differences between least squares means were tested using the PDIFF option of SAS (2001), which computes probabilities for all pair wise differences.

### 7.4. Results

The chemical composition and *in vitro* digestibility of the three forage diets is presented in Table 7.1. The differences between the forage species in terms of CP, NDF content and IVDOM% were significant (P< 0.05). Both lucerne (204 g $kg^{-1}$ DM) and *Leucaena* (191 g $kg^{-1}$ DM) had significantly higher (P< 0.05) CP contents than *Indigofera* (149 g $kg^{-1}$ DM). In contrast, *Indigofera* (577 g $kg^{-1}$ DM) forage had a significantly higher (P< 0.05) NDF content than *Leucaena* (478 g $kg^{-1}$ DM) and lucerne (438 g $kg^{-1}$ DM). However, the IVDOM% of *Indigofera* forage was significantly higher (P< 0.05) than that of *Leucaena*, but lower than that of lucerne.

The difference between forages in terms of apparent dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD) and neutral detergent fibre digestibility (NDFD) was significant (P< 0.05). *Leucaena* forage had a significantly lower (P< 0.05) DMD and OMD coefficient than either lucerne or *Indigofera* forage (Table 7.2). Apparent CPD and NDFD of *Indigofera* forage was also significantly higher (P< 0.05) than that of *Leucaena* forage (Table 7.2). However, the apparent CPD coefficient of *Indigofera* forage was significantly lower (P< 0.05) than that of lucerne.

The three forage species had significant variation in terms of g $head^{-1}$ $day^{-1}$ dry matter intake (DMI), organic matter intake (OMI) and crude protein intake (CPI), but were not

**Table 7. 1.** Chemical composition of *Indigofera*, lucerne and *Leucaena* diets fed to sheep.

| Parameter | Forage species | | |
|---|---|---|---|
| | Lucerne | *Indigofera* | *Leucaena* |
| Ash (%) | 8.8$^{a}$±(0.510) | 4.2$^{b}$±(0.51) | 7.3$^{a}$±(0.57) |
| CP (%) | 20.4$^{a}$±(0.35) | 14.9$^{b}$±(0.35) | 19.1$^{a}$±(0.39) |
| NDF (%) | 43.8$^{c}$±(0.80) | 57.7$^{a}$±(0.80) | 47.8$^{b}$±(0.89) |
| IVDOM | 67.7$^{a}$±(0.78) | 53.3$^{b}$±(0.78) | 46.2$^{c}$±(0.87) |

Means within a row followed by different superscript letter differ significantly at P< 0.05.

**Table 7. 2.** Apparent digestibility (%) of *Indigofera*, lucerne and *Leucaena* forage by sheep

| Parameter | Forage species | | |
|---|---|---|---|
| | Lucerne | *Indigofera* | *Leucaena* |
| Number of animals | 5 | 5 | 4 |
| Initial weight (kg) | 62.3$^{a}$±(5.84) | 69.3$^{a}$±(5.84) | 54.7$^{a}$± (6.53) |
| **Apparent digestibility coefficients (%)** | | | |
| Dry matter | 66.8$^{a}$±(1.41) | 63.0$^{a}$±(1.41) | 57.4$^{b}$±(1.58) |
| Organic matter | 68.0$^{a}$±(1.30) | 64.8$^{a}$±(1.30) | 58.8$^{b}$±(1.46) |
| Crude protein | 78.4$^{a}$±(2.96) | 67.9$^{b}$±(2.96) | 55.5$^{c}$±(3.31) |
| Neutral detergent fibre | 46.0$^{ab}$±(2.01) | 51.7$^{a}$±(2.01) | 43.7$^{b}$±(2.25) |

Means within a row followed by different superscript letter differ significantly at P< 0.05.

**Table 7. 3.** Voluntary intake and digestible intake of three forage species by Merino sheep.

| Parameter | Forage species | | |
|---|---|---|---|
| | Lucerne | *Indigofera* | *Leucaena* |
| **Voluntary Intake (g/head/day)** | | | |
| Dry matter | 1550.6$^{a}$±(48.01) | 1246.8$^{b}$±(48.01) | 1314.5$^{b}$± (53.67) |
| Organic matter | 1414.6$^{a}$±(48.46) | 1194.7$^{b}$±(48.46) | 1219.9$^{b}$±(54.18) |
| Crude protein | 316.1$^{a}$±(11.49) | 186.9$^{c}$±(11.49) | 251.1$^{b}$±(12.85) |
| Neutral detergent fibre | 679.4$^{a}$±(25.9) | 718.3$^{a}$±(25.9) | 628.0$^{a}$±(28.96) |
| **Voluntary Intake (g/kg W$^{0.75}$/day)** | | | |
| Dry matter | 72.8$^{a}$±(5.38) | 52.2$^{b}$±(5.38) | 67.2$^{ab}$±(6.01) |
| Organic matter | 66.4$^{a}$±(4.88) | 50.0$^{a}$±(4.88) | 62.3$^{a}$±(5.45) |
| Crude protein | 14.9$^{a}$±(1.11) | 7.8$^{b}$±(1.11) | 12.8$^{a}$±(1.23) |
| Neutral detergent fibre | 31.8$^{a}$±(2.55) | 30.1$^{a}$± (2.55) | 32.3$^{a}$±(2.85) |
| **Digestible intake (g/head/day)** | | | |
| Organic matter | 963.2$^{a}$±(43.10) | 776.3$^{b}$±(43.10) | 719.1$^{b}$±(48.19) |
| Crude protein | 248.0$^{a}$±(12.41) | 129.0$^{b}$±(12.41) | 139.9$^{b}$±(13.88) |
| Neutral detergent fibre | 313.9$^{ab}$±(20.17) | 371.0$^{a}$±(20.17) | 274.8$^{b}$±(22.55) |
| **Digestible intake (g/kg W$^{0.75}$/day)** | | | |
| Organic matter | 45.3$^{a}$±(3.42) | 32.5$^{b}$±(3.42) | 36.6$^{ab}$±(3.83) |
| Crude protein | 11.7$^{a}$±(0.88) | 5.4$^{b}$±(0.88) | 7.1$^{b}$±(0.98) |
| Neutral detergent fibre | 14.7$^{a}$±(1.51) | 15.5$^{a}$±(1.5) | 14.2$^{a}$±(1.68) |

Means within a row followed by different superscript letter differ significantly at P< 0.05.

significantly different (P> 0.05) in terms of neutral detergent fibre intake (NDFI) expressed as g head$^{-1}$ day$^{-1}$ (Table 7.3). DMI of sheep on *Indigofera* forage was equivalent to those on *Leucaena* forage, but significantly lower than that of sheep on lucerne forage (Table 7.3). The CPI was, however, significantly lower (P< 0.05) than that of both lucerne and *Leucaena* forage.

The differences between DMI and CPI per unit of metabolic body weight per day (g kg BW$^{-0.75}$ day$^{-1}$) were significant (P< 0.05). Merino sheep fed on *Indigofera* forage had similar DMI (g kg BW$^{-0.75}$ day$^{-1}$) as those sheep fed on *Leucaena* while the DMI (g kg BW$^{-0.75}$ day$^{-1}$) was significantly lower (P< 0.05) than those of sheep on lucerne. Similarly the CPI (g kg BW$^{-0.75}$ day$^{-1}$) of sheep on *Indigofera* was the least, compared to the CPI of sheep fed on lucerne or *Leucaena* forage. The digestible organic matter intake (DOMI) and digestible crude protein intake (DCPI) g head$^{-1}$ day$^{-1}$ of sheep fed *Indigofera* forage was similar as that of sheep fed *Leucaena* forage. However, the DOMI and DCPI of both *Indigofera* and *Leucaena* species were inferior compared to DOMI and DCPI of lucerne. When the comparison is done in terms of metabolic body weight, however, the DOMI (g kgBW$^{-0.75}$ day$^{-1}$) of sheep fed on *Leucaena* was similar to those sheep fed on lucerne.

**7.5. Discussion**

Nutritive value of the feed is mainly influenced by the content of structural carbohydrates, non-structural carbohydrates and the protein content and their likely interaction in the rumen (Dove 1996). According to Minson (1980), low-quality forages are considered to be those having less than 80 g CP kg$^{-1}$ DM, this being the critical level

below which voluntary intake of tropical forage is limited. All three forage species included in this study had nearly 2 to 3 fold CP levels above this threshold, and were, therefore, considered as medium to high quality forages that are able to satisfy the CP requirement of livestock ranging from mature beef cows (70 g $kg^{-1}$) to high producing dairy cows (NRC 1984; NRC 1989). Kanani *et al.* (2005) reported a CP value of 20.3% for lucerne and 27.5% for *Leucaena*. This is comparable to CP values recorded in this study for lucerne, while the value reported by Kanani *et al.* (2005) for *Leucaena* was higher than those in this study.

Forage cell walls provide the fibre that ruminant livestock require for normal rumen function. In this study, both *in vitro* and *in vivo* organic matter digestibility of *Indigofera* forage were higher than that of *Leucaena* forage. This is not in line with the lower CP content and higher NDF content of *Indigofera* forage compared to *Leucaena*. Cell wall concentrations have normally a large influence on forage digestibility and limit feed intake and digestibility (Buxton 1996). *In vivo* organic matter digestibility is a measure of energy available to ruminants and is used in protein evaluation systems (Vérité *et al.* 1987 cited Gosselink *et al.* 2004; Tamminga *et al.* 1994 cited Gosselink *et al.* 2004) to calculate rumen fermentable OM, which in turn is used to estimate rumen microbial protein synthesis. In this study, the level of *in vivo* organic matter digestibility of *Indigofera* is higher than that of *Leucaena* and equivalent to organic matter digestibility of lucerne. This indicates higher rumen fermentation and subsequently higher rumen microbial protein synthesis in *Indigofera* and lucerne than in *Leucaena* forage.

Lopez *et al.* (1998) reported that the CP digestibility was related to the CP in forage. Furthermore, San Martín and Bryant (1989) observed a protein digestibility of 61.9% in sheep for diets with 10.5% CP and the digestibility declined to 36.1% in sheep with a decrease in diet CP to less than 7.5%. These are not in agreement with the finding in this trial, which revealed higher CPD in *Indigofera* forage (67.9%) than CPD of *Leucaena* forage (55.5%), though the CP content of *Leucaena* forage was significantly higher than that of *Indigofera* forage. Others have reported apparently digestible CP of *Leucaena* forage in the range of 64.7-78% (Kharat *et al.* 1980; Upadhyay *et al.* 1974). One possible explanation is that the nitrogen in *Leucaena* may be associated with lignified cell wall to form the bulk of rumen un-degradable protein, which is unavailable for post-ruminal digestion. Cell wall degradability of the forage may also affect the overall CP digestibility.

Van Soest (1994) demonstrated that the intake of DM is negatively correlated with rumen retention time and positively correlated with ruminal volume and feed digestibility. High intake has been associated with a reduction in the extent of ruminal digestion due to decreased ruminal residence time (Staples *et al.* 1984). In this study, the differences observed in DMI and OMI g head$^{-1}$ day$^{-1}$, could be partly due to variation in retention time and partly due to variation in body weight of the experimental animals used in the study. The digestibility of DM and OM by sheep showed similar trends with up to 5-7 units lower DM and OM digestibility coefficients in *Leucaena* forage than either *Indigofera* or lucerne forage. The low levels of intake obtained with *Leucaena* are in

agreement with low to moderate (1.7-2.7% DMI as a percentage of body weight) level of voluntary intake reported elsewhere (Garcia *et al.* 1996).

The dry matter intake of sheep, as expressed both in g $head^{-1}$ $day^{-1}$ and in g $kgBW^{-0.75}$ $day^{-1}$, on *Indigofera* forage, was similar to those on *Leucaena* forage. However, the CP intake was lower than the CP intake of either lucerne or *Leucaena*. This is probably due to the lower CP content of the *Indigofera* forage used in this experiment.

The nutritive value of the forages was also considered in terms of digestible organic matter intake (DOMI). This is because it is a parameter the animals need to maximize. Digestible organic matter intake integrates both the quality and the total quantity of food ingested. The corresponding value recorded in this study for the superior forage, lucerne, is within the range described by Tainton (1999) for the species and similar to a highly palatable grass such as *Themeda triandra*, which ranges between 40-45 g kg $BW^{-0.75}day^{-1}$. On the other hand the same author reported digestible organic matter intakes of Kikuyu grass in the range of 25-35 g kg $BW^{-0.75}day^{-1}$, depending on the stage of maturity. Thus, the level of digestible organic matter intake recorded for *Indigofera* and *Leucaena* forage is slightly on the low side. Under grazing condition, a given level of digestible organic matter intake may result from a wide range of theoretically possible strategies from maximizing quality to maximizing quantity. Maximizing quality implies highly selective behavior for parts of plants or patches of high digestibility that are often of low accessibility (Baumont *et al.* 2005).

Higher DMI, OMI and CPI were observed in lucerne forage than for either *Indigofera* or *Leucaena* forage, regardless of the similarity between lucerne and *Leucaena* in terms of their chemical composition. It was suspected that the presence of secondary metabolites such as mimosine (in *Leucaena* spp) and indospicine (in *Indigofera* spp) might decrease palatability or likely reduce intake, and negatively affect digestion (Christie *et al.* 1975; Hegarty 1978, 1981; Dominguez-Bello and Stewart 1990) through various mechanisms. According to Allison (1985), animal performance is recognized as a function of feed intake, nutrient content and digestibility. Lack of significant differences between *Indigofera* and *Leucaena* forage in terms of digestible nutrient intake (DOMI, DCPI, DNDFI, etc.) means that, potentially *Indigofera* forage would likely support similar weight gains as that of *Leucaena,* but definitely lower than lucerne.

# CHAPTER 8

# CONCLUSIONS AND RECOMMENDATIONS

This investigation was focused on the domestication of *Indigofera* species, well known for its wide adaptability, palatability, tolerance to drought, salinity, flooding, etc., with the goal of generating information that will have major significance in the improvement of these species and thereby the productivity of grasslands in marginal environments. Naturally most of the legume species, adapted to the semi-arid and arid environments, have intermittent germination patterns to spread the risk of establishment failure as a result of uncertainty of the climatic conditions. Domestication of these species demands the identification of appropriate techniques that will enhance germination while minimizing the mortality of potentially viable seeds. The present study found considerable variation among the species in terms of their response to pre-planting treatment of seed. Germination was enhanced, without resulting in any risk of seed mortality, in *I. cryptantha* 7067 and *I. spicata* 8254, by scarification. In contrast, improved germination rates of *I. vohemarensis* 8730, *I. arrecta* 7524 and *I. trita* 10297 were obtained, without significant seed mortality, by immersion in boiling water. The effects of the two treatment methods are similar for both *I. brevicalyx* 7517 and *I. spicata 10299.* While either technique can be used to increase germination in the case of *I. brevicalyx* 7517, significant seed mortality may result with *I. spicata* 10299, which has a lower proportion of hard seed (54 %) than other accessions (>75%). In the latter group both techniques will improve germination but seed mortality can be as high as 40 – 50 %.

The characterization of the *Indigofera* accessions facilitates the identification of elite materials with desirable characteristics. The erect accessions were more suitable for forage production than the prostrate types, taking into consideration the associated high leaf yield and large canopy diameter for interception of rainfall as a cover crop. Morphological and agronomic characteristics, which underlie major variability in *Indigofera* accessions, have also been identified. These can be used as a core set of descriptor traits in future evaluation studies and breeding programmes. The broad trait diversity evident among the accessions of *I. spicata*, *I. arrecta* and *I. cryptantha* suggests ample opportunity for genetic improvement of those plant species through selection directly from the accessions. The grouping of accessions by phenotypic diversity alone has, however, limitations as it explains little with regard to the quality of the forage.

Nutritive value and persistency are other aspects that need to be evaluated with respect to forage crops. In this study, some of the shrubby *Indigofera* species/accessions demonstrated moderate to high biomass yields, a high crude protein concentration, a high digestibility and low indospicine concentration in the leaves. Accessions with such attributes (7850, 7598, 7592, etc) are potentially useful as protein supplements. Those accessions with more than 650 g digestible organic matter per kg DM and 188 g soluble protein content per kg DM can safely support the maintenance plus production requirements of animals (Leng, 1987). However, those accessions with high indospicine concentrations (10486, 7709, 10339, 8634, 10350, etc) are basically not fit for use as forage as the toxicity limits more efficient utilization of the forage by the animal. The remarkable variability observed both between and within species, in terms of CP,

IVDOM and indospicine concentration, provides ample opportunity for improvement through direct selection of accessions with high forage potential and feeding value for subsequent evaluation with target animals.

The seasonality of forage production is an unavoidable consequence of plant responses to a non-optimum environment (Pearson and Ison 1997). In most climates, feed is in shortest supply during autumn and winter. This makes an understanding of seasonal fluctuation in forage availability and quality an important aspect that needs to be considered in the evaluation of forage crops. Furthermore, most of the pastoral and agro-pastoral areas of Africa are categorized into semi-arid and arid ecological areas where extreme variation in climate is encountered. In these ecological areas moisture stress is the major limiting factor for the forage productivity and the existing vegetation. Therefore, adaptability to moisture deficit stress is another aspect that needs to be considered in the evaluation of forage crops targeted for moisture stressed environments and drought prone areas. In this study, the *Indigofera* species have generally shown variation in terms of their response to a range of moisture levels. Among the species evaluated *I. amorphoides* was relatively more sensitive to moisture deficit stress than *I. coerulea, I. arrecta* or *I. vicioides* with the latter being the most tolerant, considering the negative effect of moisture deficit stress on leaf area, growth, dry matter accumulation and water use efficiency. The sensitivity of a growth parameter, or plant part, appeared to depend on the inherent strategy of the species in question. General growth parameters were not as affected as leaf area and biomass allocation pattern by moisture deficit stress in *Indigofera* species. This was probably a reflection of the natural adaptability of the

species to the environmental attributes of the original collection sites, where water stress is a common phenomenon. In this study, the CP and digestible OM content of the *Indigofera* accessions were affected by season as a result of variation in climatic conditions. However, the lowest levels exhibited were still sufficiently high to consider these species as potential protein supplements in low quality diets. Compared to other conventional forage and browse species, forage from the five *Indigofera* species (*I. amorphoides*, *I. costata*, *I. cryptantha*, *I. brevicalyx* and *I. arrecta*) can be considered highly digestible. Spring growth had generally a higher quality than autumn growth, taking into consideration the high cell wall contents, and lower digestibility values and mineral concentrations (Ca, P, Mg, Zn, Mn and CP) that can arise due to fast growth rate and early maturity. Regardless of the season of growth, the *Indigofera* species could generally meet the Ca, Mg, Zn and Mn requirement of ruminant animals, while it is essential to supplement P and Cu from other sources to meet the requirements of ruminants.

Herbage quality and animal intake are closely linked, and animal intake is closely linked with production (Pearson and Ison 1997). Management practices, which produce the best compromise of matching grassland growth and quality and animal intake will maximize animal production (Pearson and Ison 1997). Within a particular class of animal, intake depends primarily on the size of and physiological state of an animal and the intake required for maintenance is directly proportional to metabolic body weight (Kleiber 1961). When an animal is productive, its requirements for energy, protein and minerals are raised further. Preston (1972) described the protein intake requirement of cattle as

1.6g digestible protein per kg metabolic body weight for maintenance purpose and an additional 5.2 g digestible protein per kg metabolic body weight for each kg of additional daily live weight gain. In this study, higher DMI, OMI and CPI were observed in lucerne forage than for either *Indigofera* or *Leucaena* forage regardless of the similarity between lucerne and *Leucaena* in terms of their chemical composition. It was suspected that the presence of secondary metabolites, such as mimosine (in *Leucaena* spp) and indospicine (in *Indigofera* spp), might decrease palatability or reduce intake, and negatively affect digestion (Christie *et al.* 1975; Hegarty 1978, 1981; Dominguez-Bello and Stewart 1990) through various mechanisms. According to Allison (1985), animal performance is recognized as a function of feed intake, nutrient content and digestibility. The lack of significant differences between *Indigofera* and *Leucaena* forage in terms of digestible nutrient intake (DOMI, DCPI, DNDFI, etc.) means that, potentially *Indigofera* forage would likely support similar weight gains as that of *Leucaena*, but definitely lower than lucerne.

To date, the outcome of this research programme has demonstrated the good agronomic potential of *Indigofera* species as an alternative forage and/or cover crop species for semi-arid and arid ecological areas. The forage was generally of high quality in terms of nutritive value, and most species, except accessions from *I. brevicalyx*, were not free from indospicine, a free amino acid injurious to the animals when fed in large quantities. However, both between and within a species, high variability was demonstrated, suggesting that there is a possibility to develop varieties that are free from indospicine, or with low levels of indospicine concentrations, from amongst the *I. arrecta*, *I. coerulea*, *I.*

*amorphoides* accessions. This research was limited in its scope, as it has involved only a limited number of germplasm, originating from Ethiopia, and the indospicine analysis is based on crude estimation. In the future, research needs to address these gaps in order to cover a wider range of *Indigofera* accessions originating from diversified countries with the aim of identifying accessions with desirable traits as forage and/or cover crops. The method used to estimate indospicine level in the forage does not discriminate precisely between arginine and indospicine, thus arginine is confounded in the estimated level of indospicine of the forage sample. Other aspects that require attention in order to realise the full potential of the *Indigofera* species include:

- Screening of more accessions of *Indigofera* germplasm for their agronomic potential, indospicine toxicity and subsequently higher nutritive value.
- Development of rapid techniques for the large-scale analysis of *Indigofera* forage samples for indospicine concentration.
- Assessing the nitrogen fixing ability of both prostrate and high yielding shrub type accessions of *Indigofera* species in the grass/legume mixture and agro-forestry system, respectively.
- Establishment of safe levels of inclusion of the forage from high yielding accessions of *I. arrecta* and *I. amorphoides* in the diet of target animals.
- Search for potential rumen microbes that would be able to detoxify the indospicine into other harmless intermediate or useful end products.

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