Characterizing Eucalyptus Camaldulensis and Eucalyptus Globulus Species Under Water Stress Conditions

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ABSTRACT

Pakistan lacks forests because of high rates of deforestation, pressure from an increasing population, and climate change. To counteract salinity, reforestation, and water logging, several eucalypt species were introduced into Pakistan. Due to Pakistan's predominantly arid and semi-arid climate, water is a scarce resource. High water content is necessary for eucalyptus to reach its full development potential. It is crucial to choose species that are suitable for low water circumstances and can flourish there. In this study, the growth behaviors of two of the most significant eucalyptus species i.e., Eucalyptus globulus and Eucalyptus camaldulensis-under water stress situations are compared. Water stress changed the growth behavior of eucalyptus species in terms of biomass allocation above and below ground. Regardless of the species, low water regimes had a significant impact on every feature assessed as the soil moisture content decreased. The number of leaves, plant height, stem diameter, root diameter, stem fresh weight, leaf moisture content, root fresh weight, root dry weight, total biomass, root biomass, and stem root ratio were all significantly lower in both species compared to the control. The ability of a plant to respond to drought is largely dependent on its root length. The roots of E. globulus, which had a shorter mean root length (17.7 \pm 4.11 cm) than the control (19.8±4.49), and E. camaldulensis grew longer than the control. For both the

control and the species, the leaf biomass was similar. Under water stress conditions, *E. camaldulensis* retained more water as evidenced by the fact that its dry weight was greatly influenced and that it lost more moisture than *E. globulus*. It is noteworthy that under stress conditions, *E. globulus* had a higher root fresh weight $(5.32\pm0.48 \text{ gm})$ than *E. camaldulensis* (4.9±0.27 gm), despite the latter having a longer root length. This suggests that the former possessed greater lateral root growth and greater root mass. Between treated and control plants, there was a substantial difference in total biomass. Comparable to *E. camaldulensis* (7.8±0.29 gm), *E. globulus* had a total biomass of 7.78±0.27 gm. The stem-root ratio was significantly impacted in both species; surprisingly, *E. globulus* exhibited a higher ratio than *E. camaldulensis*. The selection of genotypes tolerant to drought appeared to be aided by four morphological markers: root length, stem fresh biomass, stem dry biomass, and root moisture content. In comparison to the control, *E. globulus* underwent greater morphological changes in response to stress and generated biomass that was like that of *E. camaldulensis*. Consequently, it can be inferred that in situations of water deprivation and for general plantation, *E. globulus* is more adaptable than *E. camaldulensis*.

Keywords: *Eucalyptus; Water deficit; Morphological plasticity; Reforestation; Afforestation; Biomass*

INTRODUCTION

Worldwide, eucalyptus L'Hér. (Myrtaceae) is one of the most significant hardwood crops in terms of economic importance. Their highly productive woods have led to their widespread cultivation in up to 95 nations (Zhang and Wang, 2021). Brazil leads the world in productivity and planted about 9.3 million hectares of trees for industrial use in 2021 predominantly eucalyptus (80.2%) were the preferred species The majority of plantations are established by *E. grandis, E.urophylla*, and their hybrids as clonal material (IBGE, 2021). Eucalypt biomass is a common source of wood, bioenergy, fuel, charcoal, and pulp for paper manufacture because of its remarkable wood quality (Oberschelp et al., 2022). Because eucalypt plantations produce solid wood products, they help lessen the exploitation of tropical forests and help conserve biodiversity in the natural forest ecosystem (Cook et al., 2016). Pakistan introduced eucalyptus to mitigate waterlogged saline soils, and they are also a major source of pulp production. Paper consumption per capita is expected to triple in Pakistan and India over the next ten years because of rising literacy rates (Mishra, 2011). However, there isn't enough raw material to meet the demand for paper and its byproducts. Population growth and a shortage of

pulp, or raw materials, are forcing the paper industry to import the material, which is severely hurting Pakistan's economy. Agricultural waste, such as cotton lint, rice straw, kahi grass, wheat straw, wastepaper, and bagasse, is the primary source of pulp manufacturing in Pakistan. Nevertheless, as compared to imported paper goods, the quality of the paper made from these sources is lower (Zaman and Ahmad, 2012). In order to meet market demand, the development of regional pulp resources is prioritized. The primary source of high-quality and high-yield pulp is eucalyptus species, particularly *E. globulus* and *E. camaldulensis*. In several countries, including South Africa, Brazil, and Southwest Europe, wood serves as the main raw material for the production of pulp (Trabado and Wilstermann, 2008). Australia was the first country to develop the use of eucalyptus in cellulosic pulps more than fifty years ago (Ordonez and Zilli, 1971). Numerous additional eucalypt species, including *Eucalyptus nitens, E. grandis, E. saligna, E. urophylla, E. dunni*, and *E. maidenii*, are also used to make pulp products. Furthermore, enhanced eucalypt hybrids are employed in the manufacturing of pulp (Del Rio et al., 2005).

Because it can grow in a variety of environments, *E. camaldulensis* is the species that is most planted in Pakistan. Due to their high-water consumption, this species was first imported for locations that were flooded. Owing to a resurgence of interest in combating deforestation and supplying the regional pulp industry, it is currently grown in various Pakistani ecological zones. Pakistan is predominantly a semi-arid country with few water resources. Recently, eucalypts are also being planted along the canals, roadsides, and hilly areas to replace *Dalbergis sissoo* plantations due to dieback. Low water conditions caused *E. camaldulensis* to perform poorly and produce little biomass causing a lowering of the water table threatening the cultivation of agricultural crops and other vegetation. In contrast to moist areas, where volumes of up to 30 m³ per ha per year have been achieved, the wood volume produced in the drier tropical regions is typically about 5–10 m³ per ha per year on a rotation of 10–20 years (Evans, 1992). Most of the accessed terrain is found in the country's semi-arid or dry regions. There is little doubt that the use of water by eucalyptus plantations has been controversial in many regions of the world (Almeida and Soares, 2003).

Conversely, field crops like wheat, rice, cotton, and so on are planted on lands that are rich in water. There are several concerns regarding the compatibility and potential influence on local water resources when planting *E. camaldulensis* in locations that rely heavily on rainfall, owing to its high water requirements. For Pakistan to have a sustainable supply of raw materials to produce pulp, species that are appropriate for planting in arid regions must be chosen. The best

way to improve and boost pulp production in a sustainable manner is to create or choose species that can endure extended dry spells without experiencing a significant loss of biomass. The selection of genotypes with low water requirements will become increasingly important as global environmental change conditions increase and the frequency and intensity of dry spell times are predicted to increase for various parts of the world (IPCC, 2007; Rowell and Jones, 2006). According to O'Connell and Grove (1998), eucalyptus species like *E. globulus* can develop quickly in the hot, dry summer months, making them viable substitutes for *E. camaldulensis*. This study's primary goal was to determine how resistant *E. globulus* and *E. camaldulensis* species were to drought and how it affected their capacity to produce biomass.

MATERIALS AND METHODS

The study was carried out at the Forest Nursery and Experimental Area, Department of Forestry and Range Management, University of Agriculture Faisalabad, Punjab, Pakistan. *E. camaldulensis* and *E. globulus* seeds that were well-ripened, uniform in size, and open-pollinated were obtained for the trial, from the Pakistan Forest Research Institute (PFRI) in Gatwala, Faisalabad, Punjab, Pakistan.

SOIL MEDIA

The soil medium employed was homogeneous, consisting of "Bhal," a silt loam obtained by de-silting canal banks that are fed by rivers. After the soil had dried on air, it was sieved and well blended. Before being placed in the plastic trays, the organic matter and soil were combined in a ratio of 1:1. A thin layer of soil was placed over the seeds after they had been spread equally across the surface. To speed up seed germination, the plastic trays were moved into a tunnel that maintained a high humidity level and room temperature. Two-month-old seedlings were moved into earthen pots following germination. There were two stages to the experiment. The seedlings received standard watering during the first phase until they were six months old. During the second phase, regular watering was continued for the control plants while the treated plantlets were maintained at 30% water field capacity for one month to represent water stress conditions. In order to examine species growth, adaptability, and biomass partitioning, data on several biometric parameters were gathered.

BIOMASS PARTITIONING

Biomass is the total mass possessed by eucalypt seedlings in a specific unit area. Three categories—which are listed below—were used to categorize biomass production.

a. Leaf biomass allocation

Leave biomass (%) = $\frac{\text{Leave Dry Biomass}}{\text{Total Biomass}} \ge 100$

b. Stem biomass allocation

Stem biomass (%) =
$$\frac{\text{Stem Dry Biomass}}{\text{Total Biomass}} \ge 100$$

c. Root biomass allocation

Root biomass (%) = $\frac{\text{Root dry biomass}}{\text{total biomass}} \ge 100$

TOTAL BIOMASS (gm)

For calculating total biomass, the sum of all the compartment biomass was taken.

Total biomass = Leaf dry biomass + Stem dry biomass + Root dry biomass

MOISTURE CONTENT PERCENTAGE

MC% LEAVE = $\frac{\text{LEAVE FRESH WEIGHT-LEAF DRY WEIGHT}}{\text{TOTAL FRESH WEIGHT}} \times 100$ MC% STEM = $\frac{\text{STEM FRESH WEIGHT-STEM DRY WEIGHT}}{\text{TOTAL FRESH WEIGHT}} \times 100$ MC% ROOT = $\frac{\text{ROOT FRESH WEIGHT-ROOT DRY WEIGHT}}{\text{TOTAL FRESH WEIGHT}} \times 100$

STEM ROOT RATIO

Stem Root Ratio: $= \frac{\text{Stem Length}}{\text{Root Legth}} \ge 100$

STATISTICAL ANALYSIS

An analysis of variance (ANOVA) was performed on the data to ascertain how the drought affected the growth and traits of the two eucalypt species. Using the "*agricolae*" function included in the R environment, a significant mean comparison was carried out using the Duncan Multiple Range Test (DMRT) (R Core Team, 2022). Similarly, "*factoextra*" package was used to perform principal component analysis (PCA) (R Core Team, 2022).

RESULTS

Water stress had a significant effect on both species' growth (Table 1). Plant height, stem diameter, root diameter, stem fresh weight, stem moisture content, leaf fresh weight, leaf moisture content, root dry weight, total biomass, root biomass, and stem root ratio were all significantly affected in both species when compared to the control treatment (Table 1). Similarly, root lengths and leaf biomass were significantly affected and were superior under water stress than controlled plants. While *E. camaldulensis* produced the opposite results, *E. globulus* produced similar stem dry weight, leaf dry mass, root moisture content, and stem

biomass to that of the control (Table 1). When subjected to drought stress, both species produced fewer leaves than average plants. Plant height significantly decreased as predicted due to a water deficit. This decline was similar for both species (Table 1). Under control and treatment conditions, E. globulus plants were generally taller than E. camaldulensis plants. Both species' stem diameters were similarly impacted, and both displayed notable deviations from the control; however, under comparable circumstances, the diameters of the two species were comparable. The ability of a plant to respond to drought is largely dependent on its root length. Under low water regimens, the two species exhibited distinct root behaviors, with E. camaldulensis roots growing longer (24.9±9.09 inch) than E. globulus roots (17.7±4.11 inch), respectively, but neither species differed from the control (Fig. 1). On the other hand, both species' root diameters were severely impacted, while E. camaldulensis ' was more obvious than E. globulus'. In both species, there were notable differences in stem fresh weight as well. Compared to E. camaldulensis (4.85±0.39 gm), E. globulus exhibited a greater stem fresh weight $(4.96\pm0.37 \text{ gm})$ (Table 1). When the stem dry weight was determined, *E. camaldulensis* had a lower stem dry weight but E. globulus was unfazed and showed the same stem dry weight that was comparable to controlled plants (Table 1). Under low water regimens, the two species exhibited distinct root behaviors, with *E. camaldulensis* roots growing longer (24.9±9.09 inch) than E. globulus roots (17.7±4.11inch), respectively, but neither species differed from the control (Table 1). On the other hand, both species' root diameters were severely impacted, while E. camaldulensis ' was more obvious than E. globulus'. In both species, there were notable differences in stem fresh weight as well. Compared to E. camaldulensis (4.85±0.39 gm), E. globulus exhibited a greater stem fresh weight (4.96±0.37 gm). When the stem dry weight was determined, the situation was different where water stress had a considerable impact on E. *camaldulensis* but no effect on *E. globulus* (Table 1). Compared to plants that grow normally, both species produced less root biomass on average (Table 1). In both species, there were notable differences in root dry weight. In general, E. camaldulensis has a tendency to retain more moisture than E. globulus, as seen by the lower root dry weight that was observed for the leaf and stem (Table 1). The total biomass of the treated and control plants differed significantly under the same conditions, the total biomass of E. globulus (7.78 ± 0.27 gm) was similar to that of E. camaldulensis (7.8±0.29 gm). In both species, there was a significant impact on the stemroot ratio, surprisingly, E. globulus had a higher ratio than E. camaldulensis. Principal component analysis (PCA) offered an overall account of both species' phenotypic adaptation under stress (Table 2). First, two components explained 74% of the variance (Fig. 2), and four factors i.e., root length, stem fresh weight, stem dry weight, and root moisture content were significantly influenced by the species under stress (Fig. 2).

DISCUSSION

Pakistan has a small forest cover, that is not enough to cope with the local wood needs. As a result, Pakistan's economy is heavily burdened by the need to import wood and pulp products due to the country's small forest area. Salinity and waterlogging are the main issues in irrigated areas, making them unsuitable for crop cultivation (Ahmad et al., 2023). It is essential to choose tree species that can flourish in these harsh environments. Because E. camaldulensis grows quickly and requires a lot of water, it has been successfully used in places that are flooded and saline. Since *E. camaldulensis* is the primary species utilized worldwide to produce pulp, extensive plantations have been established for this purpose. There is hardly much water available in these locations. Therefore, it's critical to identify appropriate species that can flourish under these circumstances given the significance of the economy and the growing demand and burden on it. After E. camaldulensis, E. globulus is one of the most significant species for the manufacture of pulp. The water stress dramatically lowered plant growth rates, which in turn decreased most of the traits examined in both species and was consistent with other studies (Correia et al. 2013; Granda et al. 2011). The primary criteria and techniques used by euclyptus under stress conditions to prevent drought include morphological modifications such as leaf area index (LAI), vertical leaf arrangement, and rooting ability (Whitehead and Beadle, 2004). Compared to E. globulus, we found that E. camaldulensis produced longer roots in response to stress. Instead of going deep, the latter generated more root biomass to laterally collect water from the surface. After wet seasons, water stored in deep soil layers is accessed by the roots' rapid downward growth as they search for moisture in those levels. It has been noted that in E. grandis, deep roots can penetrate down to 20 m in just 5-7 years, providing access to water supplies (Christina et al., 2017). In both species, shoot biomass was considerably lower than in controlled plants, indicating a shift in resources from shoot growth to root growth. Under conditions of water stress, Matos et al. (2016) found that E. urocan had a noticeably larger root system. Stressed versus healthy plants differed markedly in the rootshoot ratio. E. microtheca species showed a decrease in the ratio of coarse to fine root mass and an increase in the root/shoot ratio (Susiluoto and Berninger, 2007). A deep taproot system and a widely dispersing lateral system located just below the soil's surface characterize eucalypt roots, which are always dimorphic. In light of the fact that dry roots were crucial for plant survival because they absorbed water during seasonal droughts, their number reduced when weather and soil depth changed. Similar to this, in hybrids of E. grandis \times E. urophylla, the vertical distribution of the fine root system varies between wet and dry times. In winter, a substantial proportion of fine roots (70 percent) were located within the upper 30 cm, however, in summer, only 30 percent were detected in the same area (Wu et al., 2023). Comparing E. globulus to E. camaldulensis, the latter generated longer roots with greater biomass, indicating greater lateral root growth that allowed *E. globulus* to draw water from both deep and surface soil. Given that *E. globulus* produced more biomass during the drought than *E. camaldulensis*, this may provide the former a further advantage. For all eucalyptus provenances, a study of the drought effect for six genotypes—three from E. camaldulensis and three from E. globulus showed a decrease in total biomass, root biomass, and leaf area (Maseda and Fernández, 2016). Moreover, genotypes with a maximum relative growth rate (RGR max) preserve leaf area and water transport capacity (leaf-specific hydraulic conductivity) during drought conditions, while genotypes with a low RGR max have a greater drop in both traits (Maseda and Fernández, 2016). The first group responds to water stress situations by retaining a high water content, exhibiting height stomatal adjustment, and significantly limiting their aerial development. Granda et al. (2014) analyzed interspecific variation amongst various E. globulus clones and observed two forms of responses. The second group presents osmotic adjustment and significantly reduces their water content because of their water usage. It continues to grow rapidly even in stressful situations. According to Valdés et al. (2013), the drought-tolerant plants had larger seeds, a more developed root system, a higher endogenous content of free and conjugated abscisic acid, and a faster rate of desiccation in the mature state. The morphological responses of seedling roots to shooting reactions in drought-stricken situations were not shown to be correlated. According to Maseda and Fernández (2016), belowground responses were not limited to resource allocation alone; they also included modifications to root morphology and anatomy. The aboveground adjustments were primarily explained by changes in biomass allocation in various parts, such as the leaf and stem. Our study found that four morphological variables-root length, root moisture content, stem fresh weight, and stem dry weight-seem to be useful indicators for eucalyptus genetic improvement targeted at water shortage tolerance. Both species' profiles of the examined physical traits were similar, and they were consistent in representing the species' adaptive behavior. Using the selected morphological criteria to analyze the effects of severe drought, it was found that E. globulus may be a superior option in dry situations with limited water supply. The two primary variables that distinguished the growth patterns of the two species were root biomass and root growth. In contrast to E. *camaldulensis*, which developed longer roots and less biomass, *E. globulus* displayed low root length and high root biomass. These traits revealed how the species behaved in terms of absorbing water and bringing the water table down. Unlike *E. globulus*, which obtains water from the upper rhizosphere, *E. camaldulensis* is known to follow the dropping water table deeper in the soil threatening surrounding vegetation and agricultural crops.

CONCLUSION

Pakistan is predominantly a country with low forest cover. Due to its need to import forest products, its economy is severely hampered. Two more significant issues that make a sizable area of land unsuitable for the development of agricultural crops are salinity and waterlogging. A few eucalyptus species were brought in primarily to fight salt and waterlogging. Because *Dalbergia sissoo* is dying off on a huge scale, eucalyptus is now being planted outside of wetlands. Because of its wide range of adaptability, *Eucalyptus camaldulensis* is the most widely planted species in Pakistan. Because *E. camaldulensis* uses a lot of water and lowers water levels, there is significant evidence against planting it in wet areas. In Pakistan, *E. globulus* is not a commonly planted species. It has been shown that in situations when there is water stress, *E. globulus* outperforms *E. camaldulensis*. *E. globulus* demonstrated similar biomass through effective morphological modification. Thus, it may be said that *E. globulus* might be a preferable plant to *E. camaldulensis* in Pakistan's low-rainfall regions.

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Trait	E. globulus	F-value	E. camaldulensis	F-value
No of leaves	Ct = 33.4±16.47	17.6***	Ct = 27.4±4.72	105.6***
	$Tr = 11.2 \pm 2.97$		Tr =11.6±1.17	
Height (cm)	$Ct = 30.5 \pm 7.80$	53.67***	Ct = 27.7±4.38	147.9***
	$Tr = 9.84 \pm 4.30$		$Tr = 9.52 \pm 1.79$	
Diameter (mm)	Ct = 2.48±0.46	66.89***	$Ct = 2.64 \pm 0.43$	99.99***
	$Tr = 1.08{\pm}0.28$		$Tr = 1.21 \pm 0.15$	
Root length (inch)	Ct =19.8±4.49	1.189 ^{N-S}	Ct =21.5±4.01	1.17N-S
	Tr =17.7±4.11		Tr =24.9±9.09	
Root diameter (mm)	Ct =1.49±0.63	16.34***	Ct =1.84±0.42	61.13***
	Tr =0.57±0.35		Tr =0.62±0.25	
Stem fresh Weight (gm)	Ct =5.6±0.57	8.89**	Ct =5.54±0.29	19.57***
	Tr =4.96±0.37		Tr =4.85±0.39	
Stem dry Weight (gm)	Ct =2.71±0.33	2.281 ^{N-S}	Ct =2.77±0.17	6.35*
	Tr =2.51±0.26		Tr =2.56±0.20	
Stem moisture content (%)	Ct =2.89±0.28	15.64***	Ct =2.77±0.28	15.14**
	Tr =2.45±0.21		Tr =2.29±0.27	
Leaf fresh weight (gm)	Ct =7.18±1.09	17.11***	Ct =7.76±0.84	48.13***
	Tr =5.71±0.23		Tr =5.77±0.33	
Leaf dry weight (gm)	Ct =3.02±0.45	3.44N-S	Ct =3.29±0.36	23.51***
	Tr =2.73±0.20		Tr =2.69±0.16	
Leaf moisture content (%)	Ct =4.16±0.69	28.61***	Ct =4.47±0.54	58.41***
	Tr =2.98±0.10		Tr =3.08±0.19	
Root fresh weight (gm)	Ct =6.34±0.59	17.67***	Ct =6.58±0.63	60.83***
	Tr =5.32±0.48		Tr =4.9±0.27	
Root dry weight (gm)	Ct =3.21±0.29	37.65***	Ct =3.34±0.25	29.38***
	Tr =2.54±0.18		Tr=2.38±0.15	
Root moisture content (%)	Ct =3.13±0.41	4.24N-S	Ct =3.24±0.25	87.58***
	Tr =2.78±0.35		Tr =2.38±0.15	
Total biomass (gm)	Ct =8.94±0.69	24.32***	Ct =9.4±0.67	49.41***
	Tr =7.78±0.27		Tr =7.8±0.29	
Stem biomass (gm)	Ct =30.34±3.34	1.87N-S	Ct =29.59±2.58	9.17**
	Tr =32.24±2.85		Tr =32.95±2.37	
Leaf biomass (gm)	Ct =33.66±3.05	1.32N-S	Ct =34.94±1.99	0.11N-S
	Tr =35.11±2.55		Tt =34.64±2.04	
Root biomass (gm)	Ct =35.99±3.28	7.51*	Ct =35.46±3.04	7.00*
	Tr =32.65±2.04		Tr =32.41±2.02	
Root shoot ratio	Ct =1.55±0.29	83.81***	Ct =1.32±0.29	78.65**
	Tr =0.55±0.19		Tr =0.45±0.25	

Table 1.	Effect of	of drought	on various	biometric	traits of E.	globulus	and <i>E</i> .	camaldulensis.
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Significant at * <0.05, ** <0.01, *** <0.001

Ct = Controlled (Watered) Tr = Water stressed

Traits	PC1 (65.4%)	PC2 (8.7%)
No of Leaves (NOL)	6.53380076	0.510020658
Height (HT)	6.70571314	0.648743877
Diameter (DIA)	7.61276684	0.194432193
Root Length (RL)	0.29237504	28.96307393
Root Diameter (RD)	6.82518512	0.052790372
Shoot Fresh Weight (SFW)	4.95813194	10.6395035
Shoot Dry Weight (SDW)	2.03954391	28.22175789
Shoot Moisture Content (SMC)	4.71037349	1.72832303
Leaf Fresh Weight (LFW)	6.99833152	0.003511202
Leaf Dry Weight (LDW)	5.64631433	0.752296206
Leaf Moisture Content (LMS)	7.23536634	0.121111139
Root Fresh Weight (RFW)	7.15246207	0.882799129
Root Dry Weight (RDW)	6.06839296	3.355526026
Root Moisture Content (RMC)	7.29024384	0.013725738
Total Biomass (TB)	7.02646681	0.047389514
Leaf Biomass (MCL)	5.36593939	2.612286329
Shoot Biomass (MCS)	2.31629927	3.014586086
Root Biomass (MCR)	0.05232442	13.63778939
Root Shoot Ratio (SRR)	5.16996882	4.600333785

Table 2. Traits contributed most significantly (bold) in response to drought stress in *E.camaldulensis* and *E. globulus*.



Figure 1. Comparison of *Eucalyptus globulus* (G1 – G10) and *Eucalyptus camaldulensis* (C1 – C10) species for root length under controlled and drought conditions. Roots grow longer mainly in *Eucalyptus camaldulensis* under drought conditions as compared to control. *Eucalyptus globulus* produced more root mass, shorter root and retain high root moisture to compensate for soil moisture deficit.



Figure 2. Biplot analysis and association of various traits in *E. camaldulensis* and *E. globulus* under drought conditions. Principal component – I explained 68.5% variance whereas PC2 (8.6%) a total of 77.1% variation was explained by both components.