

Genetic Diversity and Variability in Landraces for Key Agro-economic traits in Vegetable Roselle (*Hibiscus sabdariffa* var. *sabdariffa* L.)

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Abstract

Landraces of vegetable Roselle (*Hibiscus sabdariffa* var. *sabdariffa* L.) are under subsistence and commercial cultivation in tribal, rural and peri-urban vegetable farming systems by the tribal folks, small and marginal farmers of south India. Leaf yields of these landraces are low in farmer's conditions either due to poor production potential of landraces or poor agrotechniques. The production potential and the economic value can be enhanced by identifying the promising landraces and their intensive cultivation in the market and truck gardens. In collaboration with National Bureau of Plant Genetic Resources, Regional Station, Rajendranagar, 28 landraces of vegetable Roselle were collected from parts of Andhra Pradesh and Odisha states of India during 2010-2011. These landraces were evaluated in a randomized block design with three replications during summer 2013 at Vegetable Research Station, Rajendranagar to assess the production potential and the genetic variability for various agro-economic traits. The variation recorded within the landrace germplasm for plant height, total biomass, leaf yield, stalk yield, leaf-stalk ratio and harvest index show its potential for use in the genetic improvement. The landraces RNR-16, RNR-20 and RNR-27 were promising as indicated by the high leaf production potential of 14.22, 12.72 and 11.85 g plant⁻¹, respectively. High estimates of heritability coupled with high genetic advance as percent of mean for plant height, total biomass, leaf yield, stalk yield and leaf-stalk ratio indicating the possibility to improve these agro-economic traits through selection programs. Selection is effective for plant height, leaf yield, stalk yield, leaf-stalk ratio and total biomass in vegetable Roselle.

Keyword: Agro-Economic traits, Genetic parameters, Geographic information system, Landrace germplasm, Leaf yield, Shannon diversity index.

1. Introduction

Vegetable Roselle (*Hibiscus sabdariffa* var. *sabdariffa* L.) belongs to the family Malvaceae. It is known by different synonyms and vernacular names, such as Roselle, Indian sorrel, Jamaica sorrel, Guinean sorrel, red sorrel, Mesta and karkade (Abu-Tarboush *et al.*, 1997; Chewonarin *et al.*, 1999; Tsai *et al.*, 2002; Parkouda *et al.*, 2008). Roselle is a tetraploid ($2n=4x=72$) whose chromosomes are related to the diploid ($2n=2x=36$) *Hibiscus cannabinus* L. (Wilson and Menzel, 1964; Mclean, 1973). It is probably a native of Asia (India to Malaysia) to tropical Africa (Gomez-Leyva *et al.*, 2008). It is an important annual crop which grows successfully in the tropics and sub-tropics (Cobley, 1968). Being a tropical plant species, Roselle can be found in almost all tropical countries, such as Malaysia, South East Asia, Indonesia, Thailand and Philippines (Rao, 1996; Chewonarin *et al.*, 1999). Cultivation of the plant was reported throughout Indian subcontinent (Cobley, 1968).

It is a versatile plant similar to the coconut tree with a number of useful properties (Quezon, 2005). The juvenile or tender leaves are consumed as a green vegetable (Small and Rhoden, 1991; Delgado-Vargas and Parcedes-Lopez, 2003). It is a famous leafy vegetable crop with several uses and benefits (Ottai *et al.*, 2006). It has considerable industrial, pharmaceutical, nutritional and economic values in India and many other countries around the world. Roselle plays an important role in providing nutritional and health security and income generation and subsistence among rural farmers in developing countries (Cisse *et al.*, 2009). Being tolerant to little shade, it can be intercropped and grown in greenhouses. It is resistant to relatively high temperatures throughout the growing season (Tomes, 1990). The crop is grown mainly by traditional farming methods, exclusively under rainfed conditions (El Naim and Ahmed, 2010). It is under subsistence and commercial cultivation in tribal, rural and peri-urban vegetable farming systems by the tribal folks, small and marginal farmers of south India for its juvenile or tender leaves. There have been drastic changes in the

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production systems and marketing units of Roselle over the last few decades in south India. Presently, it has been cultivated as a monthly crop (30-40 days) with bunch of seedlings as unit of marketing in all vegetable farming systems. However, its leaf yields are very low in farmer's conditions in India due to the traditional cultivation and poor potential of cultivated varieties. As this plant has socio-economic importance, there is a constant need for the improvement of Roselle. In order to improve the leaf yield of Roselle, plant breeders should have a better understanding of the genetic diversity and variability for yield and its components. The breeding approach may lead to possible improvements in the yield and quality of the cultivars.

The cultivation and diversity of Roselle in India is unique. There are no named varieties available in this crop. A vast range of agro-climatic and socio-cultural settings in the country gave rise to a large number of Roselle landraces adapted to specific niches. A wide range of Roselle diversity on farm is under cultivation as landraces and they have long been adapted to local environments and cultural regimes, being better suited to diverse farming systems, agro-ecological niches, diverse socio-cultural settings and the needs of farmers across all of its altitudinal range. Landraces are the major characteristic of Roselle production systems in India. These landraces constitute a conspicuous source of variation for crop improvement (Zeven, 1998). It is a well-established fact that the progress in improving a crop depends on the degree of the variability in the desired character in the base material *vis-à-vis* germplasm collection. However, the genetic variability for many traits is limited in cultivated germplasm (Sabu *et al.*, 2009). Plant breeding is a process of a genetic change to improve the genetic content to a more superior genotype (variety or hybrid). Landraces or farmer varieties constitute the basic material for developing any variety or hybrid. Screening of local landraces, with the goal of identifying suitable parents for plant breeding, is the first step in the process. Development of any crop improvement program essentially depends on nature, magnitude of genetic variability, genetic advance, characters association, direct and indirect effects on yield and yield attributes (Ibrahim and Hussein, 2006). The genetic improvement of crops, for quantitative traits, requires reliable estimates of genetic variability, heritability and genetic advancement in respect to the breeding material that is presently at hand in order to plan an efficient breeding program (Dudley and Moll, 1969; Chand *et al.*, 2008). Hence, the estimates of the variability of the yield and the yield contributing characters and their heritable components in the materials are more important in any crop improvement program. The overall performance of a genotype may vary due to changes in the environment, and, if the heritability for the traits is higher, the selection process will be simpler and the response to selection will be greater (Larik *et al.*, 1997; Larik *et al.*, 2000; Soomro *et al.*, 2008). Genetic variability provides wider scope for selection. Genetic variation among selected lines is of vital importance to breeding programs that aim to produce improved cultivars for marginal growing environments (Yadav *et al.*, 2001).

The knowledge of the existing variability is essential for developing high yielding genotypes in Roselle. Agro-morphological characteristics have long been used to classify and distinguish plant genotypes.

Vegetable Roselle is largely underutilized and underexploited leafy vegetable crop. It has received no attention; not much research has been carried out on its genetic improvement, either. Little is known about its genetic potential, divergence and variability, which are supposed to be large when considering its wide geographical distribution. The level of research on Roselle does not compare to the works done on its closely related species, such as cotton (Kumar *et al.*, 1986). Most breeding of Roselle has been for its fiber yield. Roselle is endowed with a rich reservoir of genetic variability for various yield components, adaptation and quality traits (El Tahir and El Gabri, 2013). Several studies were done on genetic potential (Ibrahim and Hussein, 2006; Ibrahim *et al.*, 2013; Sabiel *et al.*, 2014) genetic diversity (El Tahir and El Gabri, 2013) and genetic variability of Roselle for calyx production as a seasonal crop (Thirthamallappa and Sherif, 1991; Gasim, 1994; Zayed *et al.*, 1996; Ibrahim and Hussein, 2006; Ahmed *et al.*, 2009; Atta *et al.*, 2011; Sabiel *et al.*, 2014) and no studies have been done on these aspects of leaf production as a monthly crop. In spite of the crop's economic prospects and importance, it has received little attention regarding its genetic improvement.

To understand and assess the value and extent of genetic variability and diversity prevalent in landraces, the present study was made on a collection of landrace accessions from two southern states of India (Andhra Pradesh and Odisha (formerly Orissa) using key agro-economic traits so as to identify the elite landraces for commercial exploitation and to identify the selection indices and breeding program for the improvement of vegetable Roselle.

2. Materials and Methods

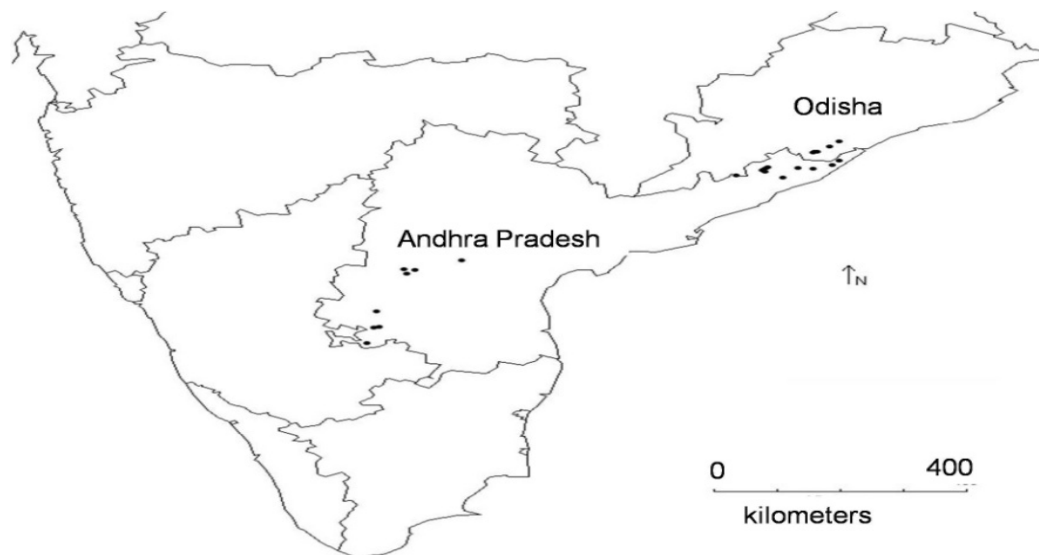
2.1. Exploration Surveys

Two exploration surveys, covering different locations of Andhra Pradesh and Odisha states of India, were conducted by National Bureau of Plant Genetic Resources (NBPGR), Regional Station, Rajendranagar in collaboration with Vegetable Research Station, Dr. Y. S. R. Horticultural University, Rajendranagar during two consecutive years 2010 and 2011. Random sampling was adopted and, whenever necessary, the sampling was based on selection; a total of 28 *Hibiscus sabdariffa* var. *sabdariffa* L. accessions, representing different agro-ecological areas in the above states was collected. Each ecotype was characterized by geographical information (name of the village, latitude, longitude and elevation). Geographical co-ordinates of the collection sites (Table 1) were recorded by Global Positioning System (Garmin 12, USA). Collection sites of the above landraces were mapped (Figure 1) Cotton bags were used for the collection of the samples. The collector number was assigned to all the accessions collected and the passport information collected was subsequently assigned with identification numbers.

Table 1. Passport data of the various landraces of vegetable Roselle

Accession ID	Collector number	Collection site						
		Geo-reference			Village	Mandal	District	State
		Latitude (°N)	Longitude (°E)	Altitude (m)				
RNR-1	SNTV-1	18.32.534	84.01.496	70	Buditi	Buditi	Srikakulam	Andhra Pradesh
RNR-2	SNTV-6	19.17.301	84.24.122	823	Raisinghi	R. Udaygiri	Gajapati	Orissa
RNR-3	SNTV-10	19.09.048	84.15.484	827	Raudiva panchayat	Lakshmpur	Gajapati	Orissa
RNR-4	SNTV-11	18.59.231	84.01.602	375	Kuttum	Gumma	Gajapati	Orissa
RNR-5	SNTV-16	18.59.861	84.02.766	733	Bubani	Gumma	Gajapati	Orissa
RNR-6	SNTV-17	19.00.283	84.04.548	733	Kingdong	Gumma	Gajapati	Orissa
RNR-7	SNTV-23	18.46.148	84.24.517	35	Peddaveedhi	Palasa	Srikakulam	Andhra Pradesh
RNR-8	SNTV-24	18.46.148	84.24.517	35	Peddaveedhi	Palasa	Srikakulam	Andhra Pradesh
RNR-9	SNTV-27	18.39.231	84.18.246	35	Nandigaon	Nandigaon	Srikakulam	Andhra Pradesh
RNR-10	SNTV-35	18.21.573	82.53.408	29	Babajipetha	Srikakulam	Srikakulam	Andhra Pradesh
RNR-11	SNTV-47	18.34.318	83.47.822	61	Palavalasa	Burgi	Srikakulam	Andhra Pradesh
RNR-12	SNTV-53	18.18.797	83.34.237	66	Chipurupalli	Chipurupalli	Vizianagaram	Andhra Pradesh
RNR-13	SNTV-58	18.27.772	83.18.857	131	Arikathota	Rambhadrapuram	Vizianagaram	Andhra Pradesh
RNR-14	SNTV-61	18.27.772	83.18.857	131	Arikathota	Rambhadrapuram	Vizianagaram	Andhra Pradesh
RNR-15	SNTV-64	18.32.465	83.19.619	131	Mettavalasa	Bobbili	Vizianagaram	Andhra Pradesh
RNR-16	SNTV-69	18.35.147	83.21.940	131	Patha Bobbili	Bobbili	Vizianagaram	Andhra Pradesh
RNR-17	SNTV-74	18.29.768	83.16.711	129	Ramabhadrapuram	Rambhadrapuram	Vizianagaram	Andhra Pradesh
RNR-18	SNT-11-7	16.04.20	78.54.22	666	Patha sunnipenta	Srisailam	Kurnool	Andhra Pradesh
RNR-19	SNT-11-9	16.04.20	78.54.22	666	Patha sunnipenta	Srisailam	Kurnool	Andhra Pradesh
RNR-20	SNT-11-10	15.48.46	78.13.20	666	Damagadha	Nandikotkur	Kurnool	Andhra Pradesh
RNR-21	SNT-11-19	15.49.40	78.03.26	280	Banddimetta	Kurnool	Kurnool	Andhra Pradesh
RNR-22	SNT-11-25	15.42.56	78.05.04	340	Namur	Orvagal	Kurnool	Andhra Pradesh
RNR-23	SNT-11-40	14.41.48	77.39.56	335	Regulakunta	Bukkarayasamudram	Anantapur	Andhra Pradesh
RNR-24	SNT-11-41	14.41.48	77.39.56	335	Regulakunta	Bukkarayasamudram	Anantapur	Andhra Pradesh
RNR-25	SNT-11-43	14.14.32	77.36.53	468	Timbaktu	Chinnakottapalli	Anantapur	Andhra Pradesh
RNR-26	SNT-11-47	14.15.59	77.41.19	442	Venkatrampalli	Chinnakothapalli	Anantapur	Andhra Pradesh
RNR-27	SNT-11-48	14.15.59	77.41.19	442	Venkatrampalli	Chinnakothapalli	Anantapur	Andhra Pradesh
RNR-28	SNT-11-55	13.49.57	77.30.01	634	Hindupur	Hindupur	Anantapur	Andhra Pradesh

RNR= Rajendranagar Roselle

**Figure 1.** DIVA-GIS mapping of collection sites of landraces of vegetable Roselle from Andhra Pradesh and Odisha

2.2 Pre-breeding

It is short day plant having critical photoperiod of 10-12.5 hours (Hacket and Carolone, 1982). Since natural cross-pollination is reported in Roselle (Sanyal and Dutta, 1954), selfing must be done to maintain the genetic purity of the diverse germplasm accessions, which would be in a close proximity in a breeding nursery. These landraces were pre-bred by selfing during rainy season 2012 at Vegetable Research Station, Rajendranagar for initial seed increase for further evaluation and conservation. These landraces were christened with accession identification numbers (accession IDs starting with RNR; RNR-1 to RNR-28).

2.3. Experimental Design and Agro-Techniques

Twenty-eight landraces were utilized for the present study. The experiment was conducted at the Experimental Farm of the Vegetable Research Station, Rajendranagar, Hyderabad, Andhra Pradesh, India (latitude 17.19°N and longitude 79.23°E, and altitude 222 m above mean sea level) during summer, 2013. The experimental design was a randomized block design with three replicates. The treatment plot (block) consisted of 28 rows (1 row per genotype). The individual plot was 1.0 m long and 0.20 m wide. In each replication, a plant population of 20 plants per row, plot and genotype was maintained. Three seeds per hill were dibbled with an intra-row spacing of 0.05 m and an inter-row spacing of 0.20 m under high density planting system. Thereafter hills were thinned to one plant at two weeks after sowing. All agronomic practices were maintained for a whole duration of the experiment. Regular plant protection measures were carried out to safeguard the crop from pests and diseases.

2.4. Recording of Biometric Data

All landraces were subjected to harvest at once by pulling out the seedlings along with the root system reasonably intact 40 days after sowing as practiced by farmers under once-over harvest system. Crop plots were judiciously irrigated at the previous night. Four out of six variables were measured on the seedling plants of the 28 accessions of Roselle. Five seedling plants chosen randomly from middle of rows of each plot were tagged for measurements on plant height (cm), total biomass (g/plant), leaf yield (g/plant) and stalk yield (g/plant). Height of the plant (cm) from ground level to tip of the main stem was measured in centimeters just before the harvest on the tagged seedling plants. A graduated scale of 100 cm long was used to measure the plant height. These tagged seedling plants were pulled out along with root system reasonably intact. These sampled seedling plants were weighed with a digital analytical balance (± 0.001 g) to arrive at the seedling weight or the total biomass (g/plant) consisting of above ground shoot system and below ground root system. These sampled seedling plants were stripped off leaves to facilitate recording of data on separated leaves and stalks to arrive at leaf yield (g/plant) and stalk yield (g/plant), separately. Leaf yield (g/plant) and stalk yield (g/plant) were recorded on a digital analytical balance (± 0.001 g). The remaining two variables viz., leaf-stalk ratio and harvest index were calculated using replicated mean values of leaf

yield, stalk yield and total biomass. Leaf-stalk ratio was calculated as the ratio of leaf yield (g/plant) and stalk yield (g/plant). The harvest index was taken as the ratio of the leaf yield (g/plant) to total biomass (g/plant).

2.5. Statistical Analysis

The replicated means of each individual landrace was employed in the statistical analysis. The data, thus recorded, were subjected to the analysis of variance (Steel and Torrie, 1980). Tests for the significant difference between the means were made using the procedure of analysis of variance (ANOVA) and the Student Newman Keuls test at 0.05 and 0.01 probability levels. A statistical analysis for estimating the mean performance of landraces and major genetic components for six agro-economic traits was performed using SAS Enterprise Guide Version 4.2 (SAS Institute Inc., 2009). From the analysis of variance, the phenotypic, the environmental and genotypic components of variance were estimated as follows, as per the formula suggested by Lush (1940):

$$\text{Environmental variance} = \sigma_e^2$$

$$\text{Genotypic variance } (\sigma_g^2) = \frac{Mg - Me}{r}$$

Where,

Mg = mean sum of squares of genotypes (treatments)

Me = mean sum of squares of error

r = number of replications (blocks)

Phenotypic variance (σ_p^2) = Genotypic variance (σ_g^2) + Environmental variance (σ_e^2)

The analysis of variance also permits the estimation of phenotypic and genotypic coefficients of variation (Burton, 1952). The Genotypic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV) were computed by adopting the method of Burton (1952) and Burton and de Vane (1953). The PCV and GCV values were classified as low (<10%), moderate (10-20%) and high (>20%) as suggested by Sivasubramanian and Menon (1973):

$$\text{Phenotypic coefficient of variation (PCV)} = \frac{\sqrt{\sigma_p^2}}{\bar{X}} \times 100$$

where,

PCV = phenotypic co-efficient of variation

\bar{X} = general mean

σ_p^2 = phenotypic variance

$$\text{Genotypic coefficient of variation (GCV)} = \frac{\sqrt{\sigma_g^2}}{\bar{X}} \times 100$$

where,

GCV = genotypic co-efficient of variation

\bar{X} = general mean

σ_g^2 = genotypic variance

The broad sense heritability (h_{bs}^2) was estimated for all characters as the ratio of genotypic variance to total or phenotypic variance (Lush, 1940). The heritability values were classified as low (<30%), moderate (30-60%) and high (>60%) as suggested by Johnson *et al.* (1955):

$$h^2 = \frac{\text{Genotypic variance}}{\text{Phenotypic variance}} \times 100$$

The estimates of genetic advance (GA) at 5% selection intensity (2.06) and genetic advance as per cent of mean were obtained using the procedure given by Allard (1999). The estimates of the genetic advance and the genetic advance as percent of mean were classified as low (<10%), moderate (10-20%) and high (>20%), as suggested by Johnson *et al.* (1955):

$$GA = h^2_{bs} \times \sigma_p \times K$$

where, h^2_{bs} = Heritability estimate in broad sense

σ_p = Phenotypic standard deviation of the trait

K = Standard selection differential which is 2.06 at 5 per cent selection intensity

$$GA \text{ as per cent of mean} = \frac{GA}{\text{Grand mean}} \times 100$$

A hierarchical clustering was carried out following Ward's minimum variance method (Ward, 1963). DIVA-GIS version 7.5.0, free downloadable software was used for mapping of the collection sites of the above landraces and analysis of diversity of the collected landrace accessions of Roselle and for generating grid maps (Figure 2). Shannon diversity index was calculated for all the agro-economic traits with observed data using DIVA-GIS version 7.5.0 free downloadable software (Hijmans *et al.*, 2012).

3. Results

3.1. Analysis of Variance

Analysis of variance (Table 2) revealed significant differences ($P < 0.01$) among 28 genotypes of Roselle for all of the six agro-economic traits *viz.*, plant height, leaf yield, stalk yield, leaf stalk ratio, harvest index and total biomass under study. Significant differences were not observed within replications for almost all traits.

Table 2. Analysis of variance for various agro-economic traits of vegetable Roselle

Character	Mean squares		
	Replications (2)	Genotypes (27)	Error (54)
Plant height (cm)	1.1074	240.9060**	6.0603
Total biomass (g/plant)	2.7955	120.9715**	2.0893
Leaf yield (g/plant)	0.6908	37.4995**	0.5284
Stalk yield (g/plant)	0.7482	25.1438**	0.5639
Leaf-stalk ratio	0.0654	1.1004**	0.0289
Harvest index	0.0007	0.0149**	0.0003

**Significant at $P \leq 0.01$ level

Values in parentheses denote degrees of freedom.

3.2. Production Potential of Landraces

The ranges of mean values revealed a sufficient variation for all the traits under study (Table 3). Large variations between ecotypes were observed for all the traits. A wide range of variations was observed in most of the agro-economic traits. Plant height, total biomass, leaf yield, stalk yield, leaf stalk ratio and harvest index (Table 3) ranged from 16.93-51.23 cm, 3.16-23.66 g/plant, 1.95-

14.22 g/plant, 0.8-10.94 g/plant, 1.18-3.25 and 0.54-0.77, respectively. In the landrace germplasm used in the present study, a maximum range of variability (Table 3) was observed for plant height (34.30 cm) followed by total biomass (20.50 g/plant) and leaf yield (12.27 g/shoot). RNR-20 (51.23 cm) had tallest plants followed by RNR-14 (45.87 cm) and RNR-25 (43.07 cm). RNR-20 (23.66 g) had highest total biomass followed by RNR-16 (23.55 g) and RNR-10 (19.91 g). RNR-16 (14.22 g) had highest leaf yield followed by RNR-20 (12.72 g) and RNR-27 (11.85 g). RNR-20 (10.94 g) had highest stalk yield followed by RNR-16 (9.33 g) and RNR-10 (8.74 g). RNR-9 (3.25) had highest leaf-stalk ratio followed by RNR-7 (3.05) and RNR-26 (2.74). RNR-9 (0.77) had highest harvest index followed by RNR-7 (0.76) and RNR-3, RNR-5, RNR-22 and RNR-26 (0.72). On the basis of genetic potential for leaf yield (Table 2), the landraces RNR-16 (14.22 g/plant), RNR-20 (12.72 g/plant), RNR-27 (11.85 g/plant), RNR-10 (11.17 g/plant) and RNR-25 (10.50 g/plant) were promising from consumer point of view.

Table 3. Mean performance of landraces of vegetable Roselle for agro-economic traits

Accession number	Plant height (cm)	Total biomass (g/plant)	Leaf yield (g/plant)	Stalk yield (g/plant)	Leaf-stalk ratio	Harvest index
RNR-1	33.00	7.23	4.73	2.50	1.91	0.65
RNR-2	20.42	3.27	2.30	0.97	2.39	0.71
RNR-3	22.70	4.35	3.12	1.23	2.54	0.72
RNR-4	37.30	8.08	5.31	2.77	1.92	0.66
RNR-5	20.27	3.96	2.82	1.14	2.52	0.72
RNR-6	24.58	8.20	5.57	2.64	2.11	0.67
RNR-7	23.30	7.44	5.61	1.83	3.05	0.76
RNR-8	25.50	10.28	6.85	3.44	1.99	0.66
RNR-9	16.93	3.38	2.57	0.80	3.25	0.77
RNR-10	41.30	19.91	11.17	8.74	1.28	0.56
RNR-11	29.40	6.51	3.65	2.87	1.28	0.56
RNR-12	32.50	8.45	4.63	3.83	1.23	0.55
RNR-13	32.17	7.90	4.37	3.53	1.24	0.56
RNR-14	45.87	16.48	9.12	7.36	1.24	0.55
RNR-15	35.33	13.81	8.51	5.31	1.62	0.61
RNR-16	38.53	23.55	14.22	9.33	1.53	0.61
RNR-17	31.10	13.94	8.28	5.67	1.46	0.59
RNR-18	24.27	6.07	4.23	1.83	2.33	0.69
RNR-19	24.63	7.77	5.48	2.29	2.45	0.71
RNR-20	51.23	23.66	12.72	10.94	1.18	0.55
RNR-21	31.40	3.98	2.43	1.55	1.57	0.61
RNR-22	21.93	4.20	2.99	1.21	2.49	0.72
RNR-23	20.50	3.59	2.50	1.09	2.29	0.70
RNR-24	21.53	3.16	1.95	1.21	1.62	0.61
RNR-25	43.07	18.15	10.50	7.65	1.37	0.58
RNR-26	19.58	3.63	2.65	0.99	2.74	0.72
RNR-27	38.23	18.33	11.85	6.49	1.83	0.64
RNR-28	34.37	9.88	5.34	4.54	1.18	0.54
S. Ed	2.01	1.18	0.59	0.61	0.14	0.01
CV	8.20	15.04	12.31	20.27	8.87	2.62
CD (5%)	4.03	2.36	1.19	1.23	0.28	0.03

RNR= Rajendranagar Roselle

3.3. Genetic Diversity Analysis

The DIVA-GIS grid maps, generated for Shannon diversity index in Roselle (Figure 2), indicated that the Andhra Pradesh is an important pocket for collecting the diversity of Roselle. Shannon diversity index (Table 4) varied from 0.389 to 2.00 for plant height, stalk yield, total biomass and leaf yield, 0.358 to 2.00 for leaf-stalk ratio and 0.322 to 2.00 for harvest index suggests existence of significant variability among the landraces.

Table 4. Shannon diversity index for various key agro-economic traits of vegetable Roselle

Agro-economic trait	Shannon diversity index range
Plant height (cm)	0.389 - 2.00
Total biomass (g/plant)	0.389 - 2.00
Leaf yield (g/plant)	0.389 - 2.00
Stalk yield (g/plant)	0.389 - 2.00
Leaf stalk ratio	0.358 - 2.00
Harvest index	0.322 - 2.00

A dendrogram (Figure 3), illustrating the genetic divergence of landraces, was generated following Ward's

minimum variance method using semi-partial R^2 values. A cluster analysis, based on semi-partial R^2 values, is shown in Figure 3. The dendrogram constructed revealed two major clusters (cluster-I and cluster-II). The first major bifurcation in the dendrogram (Figure 3) separated the 28 accessions into two major clusters (cluster-I and cluster-II). Clusters II could be further divided into sub-clusters (cluster-IIA and cluster-IIB). Cluster I consisted of six accessions, while cluster IIA and cluster IIB consisted of 13 and 9 landraces, respectively. The genotypes of the sub-cluster-IIA showed high genetic distance than the sub-cluster-IIB. The multiple accessions collected from single collection sites like Peddaveedhi (RNR-7 and RNR-8), Arikathota (RNR-13 and RNR-14), Patha sunnipenta (RNR-18 and RNR-19), Regulakunta (RNR-23 and RNR-24) and Venkatrampalli (RNR-26 and RNR-27) formed separate groups. Further, certain Roselle accessions, from different collection sites, were clustered together; for example RNR-11 from Palavalasa and RNR-21 from Bandimetta in one group and RNR-10 from Babajipetha and RNR-25 from Timbaktu in another group. There are several duplications out of the 29 accessions collected.

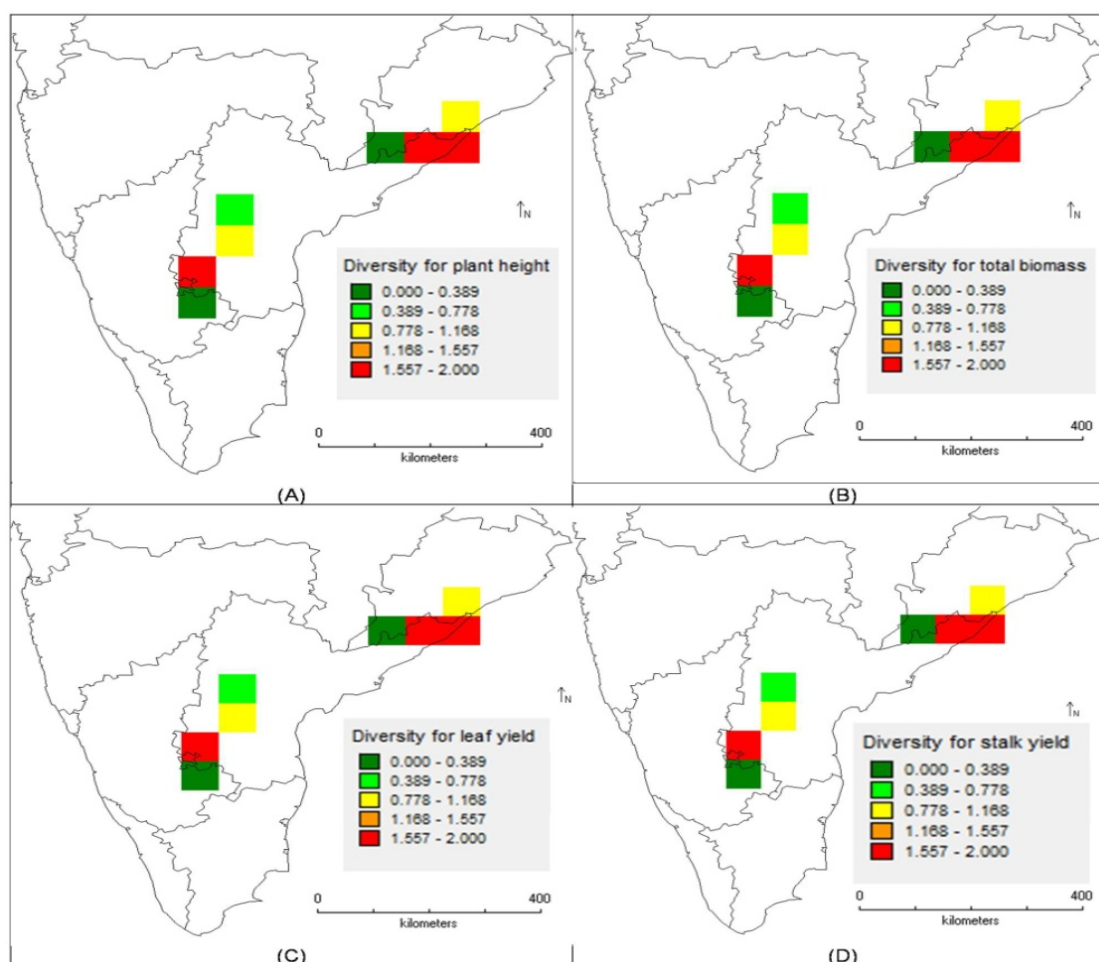


Figure 2. Shannon diversity index for (A) plant height; (B) total biomass; (C) leaf yield; (D) stalk yield of vegetable Roselle landraces

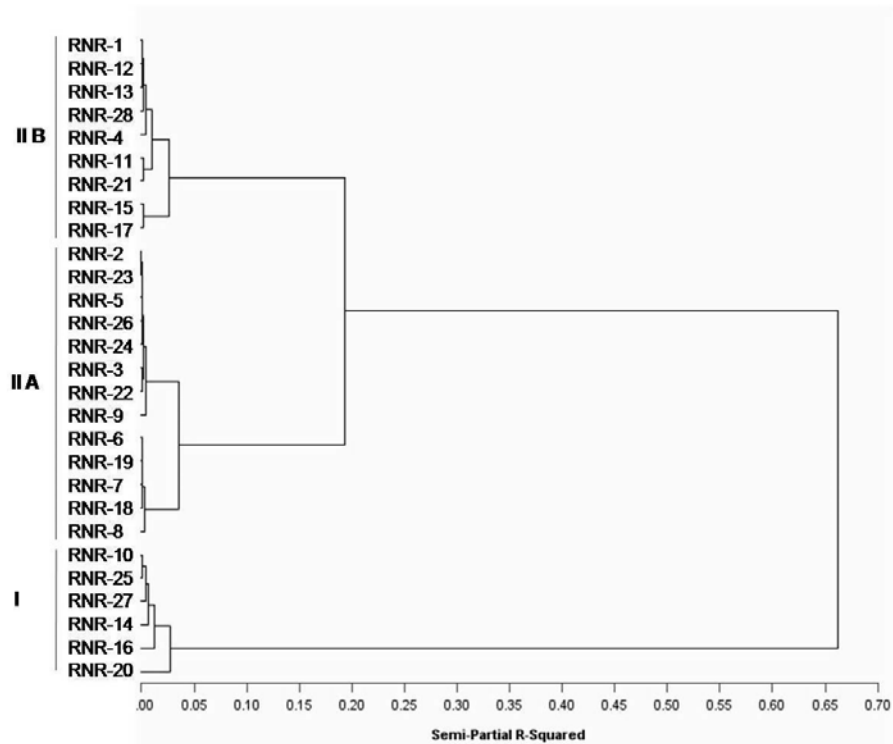


Figure 3. Ward's minimum variance dendrogram showing clustering of landraces of vegetable Roselle

3.4. Genetic Variability Analysis

In general, phenotypic variances were higher than the corresponding genotypic variances for all the characters under study, except for the harvest index (Table 5). The phenotypic variance was the highest for plant height (84.34) followed by total biomass (41.72) and leaf yield (12.85). Similarly, the genotypic variance was the highest for plant height (39.63), followed by total biomass (41.72) and leaf yield (12.32). The degree of variability, shown by different parameters, can be judged by the magnitude of GCV and PCV. PCV showed that the extent of genetic variability in the population ranged from 11.18 (harvest index) to 79.87 (stalk yield). GCV showed that the extent of genetic variability in the population ranged from 10.84 (harvest index) to 77.25 (stalk yield). The estimates of PCV (Table 5) were the highest for stalk yield (79.87%) followed by the total biomass (67.18%) and leaf yield (60.67%), while the lowest for the harvest index (11.18%) followed by plant height (30.58%) and leaf-stalk ratio (32.44%). The estimates of GCV (Table 5) were the highest for stalk yield (77.25%), followed by the total biomass (65.48%) and leaf yield (59.41%), while the

lowest for the harvest index (10.84%), followed by plant height (29.46%) and leaf-stalk ratio (31.20%). The estimates of PCV (Table 4) were of a high magnitude (>20%) for almost all the traits except for the harvest index with a moderate magnitude (10-20%). The estimates of GCV (Table 5) were of a high magnitude (>20%) for almost all the traits except for the harvest index with moderate magnitude (10-20%). In general, the magnitude of PCV was higher than the corresponding GCV for all the six characters under study (Table 5). The magnitudinal differences between the estimates of GCV and PCV were the highest for stalk yield (2.62), followed by leaf yield (1.26) and leaf-stalk ratio (1.24). The estimates of heritability (Table 5) were of a high magnitude (>60%) for almost all the traits except for the harvest index with a moderate magnitude (30-60%). The estimates of genetic advance as percent of mean (Table 5) were of a high magnitude (>20%) for all the traits under study. High estimates of heritability (>60%), coupled with a high genetic advance as percent of mean (>20%), were observed for almost all the traits except for the harvest index.

Table 5. Genetic parameters for various agro-economic traits in vegetable Roselle

Character	Variance		Coefficient of variation (%)		Heritability (%)	Genetic advance (%)	Genetic advance as per cent of mean
	Phenotypic	Genotypic	Phenotypic	Genotypic			
Plant height (cm)	84.34	78.28	30.58	29.46	0.93	17.56	58.47
Total biomass (g/plant)	41.72	39.63	67.18	65.48	0.95	12.64	131.46
Leaf yield (g/plant)	12.85	12.32	60.67	59.41	0.96	7.08	119.84
Stalk yield (g/plant)	8.76	8.19	79.87	77.25	0.94	5.70	153.93
Leaf-stalk ratio	0.39	0.36	32.44	31.20	0.93	1.18	61.82
Harvest index	0.01	0.01	11.18	10.84	0.41	0.14	21.67

4. Discussion

In general, species and cultivars are chosen according to their fitness for local conditions. *Hibiscus sabdariffa* has an extensive intraspecific variation, a differentiation into two botanical varieties (sabdarriffa and altissima) and a further differentiation into different cultivar groups as landraces. Landraces are most often heterogeneous with a blend of different individual plants maintained by farmers in a local environment and constitute a significant portion of the cultivated crop gene pool. Landraces are the heterogeneous crop populations (Harlan, 1975) which have not originated due to modern plant breeding (Louette, 2000) and constitute a significant source of diversity in the crop gene pools. The selection imposed by farmers makes landraces significant with their social, cultural and religious dimensions in farming. Landraces are crop populations, selected and maintained by farmers within the natural system of evolution (Allard, 1999). They are passed from one generation of farmers to another generation and are exposed to natural and human selections in a local environment. Landraces are known to be resistant to several biotic/ abiotic stresses, nutritionally diverse and considered as valuable genetic resources in crop improvement. These landraces are the only resource available in a resource-poor environment and this genetic variation could be exploited in plant breeding where access to new technology is difficult (Witcombe, 1999). A range of diversity in the form of vegetable Roselle (*Hibiscus sabdariffa* var. *sabdarriffa* L.) landraces is under cultivation by the farmers in open fields in traditional leafy vegetable production and management systems of India. Roselle, planted by traditional farmers in India, is usually of different types and cultivars. These landraces are grown for diverse uses such as for home consumption and sale and are adapted to a range of agro-ecological niches. Such diverse genetic resources of Roselle in India are threatened by different factors, including environmental and production factors. Their utilization in the crop improvement programs depends largely on their availability, genetic diversity and variability. The collection and the characterization of local landrace germplasm have become necessary. Though, the use of the changing land pattern and the habitat destruction have led to the genetic erosion of landraces of Roselle, the collected and reserved gene serves as a natural genetic resource for crop improvement programs of Roselle.

4.1. Production Potential of Landraces

In general, the leafy vegetable crop production and the marketing systems are interdependent. Various kinds of leafy vegetable crop production and marketing systems have evolved over the years in south India. Being an annual crop (Berhaut, 1979), it is traditionally cultivated as a seasonal crop (150-180 days) with periodical leaf pickings or periodical stem cuttings under low density planting (40-30 × 40-30 cm) and medium density planting (30-20 × 30-20 cm) systems, respectively. The low density planting system, with periodical leaf pickings in which 'heap of tender leaves' as a unit of marketing, was practiced up to 1980s. The plants may be stripped off leaves 6 weeks after sowing leaving the stalks in the field

with subsequent leaf pickings at 4 weeks interval. The medium density planting system, with periodical stem cuttings in which 'bunch of tender stems' as a unit of marketing, was practiced in 1990s. The plants may be cut off 6 weeks after sowing leaving only 7.5-10 cm of stem in the field with subsequent cuttings at 4 weeks interval. The high density planting with once over harvest, in which 'bunch of seedlings' as a unit of marketing, became more common in 21st century. Currently, it is cultivated as a monthly crop (30-40 days) under a high density planting and once over harvest system in all vegetable farming systems. Under this high density planting and once over harvest system, vegetable Roselle is grown either by broadcasting or drilling at a spacing of 10-20 × 5-10 cm so as to harvest 500000-2000000 plants per hectare just 30-40 days after sowing depending on variety and plant type. In the present study, the production potential of 28 landraces of vegetable Roselle under a high density planting and once over harvest system was discussed.

The analysis of variance revealed significant differences ($P < 0.01$) among 28 genotypes of Roselle for all of the six agro-economic traits, indicating the presence of a significant amount of variability for effective selection. Similar results were reported by many workers (Ibrahim and Hussein, 2006; Ibrahim *et al.*, 2013) indicating that the diversity can be attributed to genetic as well as their interaction with the environment (Kooras, 1987; Thirthamallappa and Sherif, 1991; Zayed *et al.*, 1996). In the germplasm, used in this study, a maximum variability (Table 3) was observed for plant height (34.30 cm), followed by total biomass (20.50 g/plant) and leaf yield (12.27 g/shoot). The ranges of mean values revealed a sufficient variation for all the traits under study. Large variations among landraces were observed for plant height, total biomass, and leaf yield. Such large variation indicated the scope for improving the population for these characters. A great variability among landraces was observed in leaf yield, indicating the possibility to increase leaf production through selection.

In conventional breeding, the choice of parents is based on a high mean performance; out of the six quantitative traits under study, high mean values are desirable for plant height, total biomass, leaf yield, stalk yield, leaf-stalk ratio and harvest index. Hence, the breeders are in absolute need of a desirably high or low mean value, depending upon the character, which is considered a main criterion for effective selection forever. In Roselle, of the six quantitative traits under study, high mean values are desirable for plant height, total biomass, leaf yield, stalk yield, leaf-stalk ratio and harvest index. In the present study, significant differences in plant height were observed among the landraces. Ibrahim and Hussein (2006) also observed significant differences among genotypes of Roselle for plant height. Short plants are often preferred in breeding programs, because they can reduce the lodging problems and responds in a better way to fertilizers. However, plants with longer main stems are stronger and do not fall easily in production levels compared to short plants in Roselle (Chang *et al.*, 2006). Shorter plants with due reduction in internodal length adds the more number of leaf production points in plant. The leaf biomass percentage decreased as the Roselle

plant increased in height. There was advantage of harvesting maximum leaf yields from tall Roselle plants.

Stalk and leaf yields have been currently referred to in terms of yield per plant. Both of these references are usually made on a fresh weight basis. Typically, the leaf and stalk yields will increase throughout the growing season, with the rate of growth reduced by drought, decreasing heat units, and flowering. Leaf production continues throughout the growing season, and is reduced by the same factors, but unlike stalk yields, leaf yields will often increase only during the first half of the growing season and level off or even decrease in the second half of the season. The leaf production continues, but, as a result of leaf abscission, the older lower leaves will drop off, as the plant grows taller. The loss of the lower older leaves limits leaf yields, and results in a decreasing leaf percentage on the whole-plant basis. The leaf yields and total biomass (plant weight) are also important considerations when selecting cultivars for leaf production, because the leaves are the primary source of protein (Webber, 1993a). Researchers reported the differences among cultivars for stalk and leaf biomass percentages (Webber, 1993a and 1993b). These stalk and total biomass can also be greatly affected by plant maturity at harvest. The high leaf-stalk ratio and the harvest index are the key aspects. The harvest index is not directly a yield contributing trait but is considered an important parameter for the genetic improvement of genotypes. It is normally accepted within the Roselle industry to report plant yields on total biomass, leaf yield, leaf-stalk ratio and harvest index. It is much more important to consider the leaf yield when discussing plant yields.

On the basis of genetic potential for leaf yield (Table 3), accessions RNR-16 (14.22 g/plant), RNR-20 (12.72 g/plant) and RNR-27 (11.85 g/plant) were found promising owing to their high yielding potential under high density planting and single harvest system. These genotypes can be evaluated for their stability in various ecological zones across the state of Andhra Pradesh. Upon assessing yield stability through multi-location trials, these genotypes may be used for large-scale cultivation if found suitable. These landraces, with a high leaf yield potential, could be used as donor parents for improvement of indigenous varieties for higher leaf productivity in Roselle improvement programs. The landraces were ranked by the breeders; the adoption rate should be maximal if these local varieties keep the same performance in the farmer's fields. These promising landraces are to be disseminated using on-farm conservation in some sites in the state and at Research Stations. These local varieties of Roselle could be tapped and used in the breeding program, which necessitates the on-farm maintenance of landraces. In order to increase the adoption rate of the promising landraces of Roselle, a participatory selection, on the promising landraces based on users' criteria preferences, should be carried out at the Research Stations, with heavy involvement of the users (producers, consumers and traders). The identification of the promising Roselle landraces will enable the Roselle industry to further move forward in terms of providing choices for varietal selection. By promoting the raising of

these promising landraces of this underutilized species, extension workers can help diversify farming systems throughout the tropics, thereby increasing food and economic security on marginal lands.

4.2. Genetic Diversity of Landraces

A better understanding of the genetic diversity distribution is essential for its conservation and use. It will help us in determining what to conserve and where to conserve, and will improve our understanding of the taxonomy and the origin and evolution of the plant species of interest. The distribution of Roselle diversity varied across locations and states (Figure 2). Shannon diversity index (Table 4) varied from 0.389 to 2.00 for plant height, stalk yield, total biomass and leaf yield, 0.358 to 2.00 for leaf-stalk ratio and 0.322 to 2.00 for the harvest index suggesting the existence of a significant variability among the landraces. DIVA-GIS grid maps, generated for Shannon diversity index for Roselle (Figure 2), indicated that Andhra Pradesh and Odisha states had important pockets of diversity in Roselle. The study showed a greater diversity in Andhra Pradesh followed by Odisha, indicating that earlier is important habitat for an on-farm conservation. DIVA-GIS has been used successfully in assessing biodiversity and in identifying areas of high diversity for Phaseolus bean (Jones *et al.*, 1997), wild potatoes (Hijmans *et al.*, 2000), Piper (Parthasarathy *et al.*, 2006), horse gram (Sunil *et al.*, 2008), and black gram (Abraham *et al.*, 2010). However, there is a need for measuring the impact on the change of genetic diversity over time both at village and landscape levels.

The genetic divergence analysis estimates the extent of diversity among the selected genotypes. A dendrogram (Figure 3), illustrating genetic divergence of landraces, was generated following Ward's minimum variance method using semi-partial R^2 values. A multivariate analysis, following Ward's minimum variance method, revealed that the landrace germplasm of Roselle under study seems to be quite diverse agronomically, with a high level of divergence among the accessions under study. The diversity in Roselle germplasm could be attributed to the fact that the studied germplasm represented local farmers' cultivars that are used for commercial production in India, rather than improved advanced varieties. The multivariate analysis, following Ward's minimum variance method, revealed distinct clustering pattern (Figure 3). The present evaluation shows the existence of an intra-specific diversity of the vegetable Roselle studied. The dendrogram constructed by the UPGMA clustering method also revealed the genetic relationship and demonstrated a considerable divergence among 16 accessions of Roselle and kenaf (Omalsaad *et al.*, 2014). The diversity assessment of a collection of 124 roselle accessions and 16 accessions of its close relatives *Hibiscus cannabinus* and *Abelmoschus esculentus* based on ten agro-morphological traits identified two major distinct groups in *H. sabdariffa* using a Bayesian method wherein these two genetic groups were associated with statistical differences for three phenological characteristics: number of days to flowering, 100-seed weight and calyx size (Bakasso *et al.*, 2014). The genotypes of the sub-cluster-IIA showed a higher

genetic distance than those of the sub-cluster-IIB, which clearly indicates the genetic closeness of the groups of Roselle landraces. In the breeding program, the distantly related genotypes should be of a great interest to get the desirable segregates with wide genetic traits. Roselle is a self-pollinating crop species. Flowers being cleistogamous, pollination occurs naturally in the bud stage before the flower blooms. This phenomenon has become a barrier to natural or artificial hybridization to produce genetic variation, and, hence the reason why the breeding program is rarely carried out in a conventional manner. Roselle is a tetraploid ($2n = 72$) species and, therefore, their segregating populations need a longer time for purification compared to the diploid species. Furthermore, Roselle has cleistogamous flowers. Thus, crop improvement through conventional hybridization is very difficult to be carried out (Jain, 1979; Vaidya, 2000). To avoid these limitations, mutation breeding is recommended to generate a new source of genetic variability. The scoring matrix should be used. The determination of the nature and degree of the genetic diversity of accessions is extremely important to the plant breeder in choosing the diverse parents for a purposeful hybridization in the breeding of crop plants. Hence, it is indispensable that the natural genetic diversity of Roselle for crop improvement be scrutinized and exploited.

The clustering pattern revealed that the landraces, collected from different collection sites, are grouped into different clusters with certain exceptions. For example, the multiple accessions collected from single collection sites like Peddaveedhi (RNR-7 and RNR-8), Arikathota (RNR-13 and RNR-14), Patha sunnipenta (RNR-18 and RNR-19), Regulakunta (RNR-23 and RNR-24) and Venkatrampalli (RNR-26 and RNR-27) formed separate groups. These studies showed that accessions from the same geographical region may differ genetically and phenotypically as well as in their adaptability. Further, certain Roselle accessions, from different collection sites, were clustered together; for example RNR-11 from Palavalasa and RNR-21 from Bandimetta in one group and RNR-10 from Babajipetha and RNR-25 from Timbaktu in another group. The pattern of clustering did not show any distinct relationship with the collection site of landraces. This indicates that the geographic diversity is not the only factor in determining the genetic diversity. Differences of genetic differentiation was probably associated with the differences in the sampling methods and the accession handling. The genotypes with the same geographic origin could have undergone a change in different characters under the selection during the process of evolution. The free clustering of the landraces suggested the influence of the direction of the selection pressure for realizing a maximum yield in different ecosystems; the nicely evolved homeostatic devices would favor the constancy of the associated characters and, thus, show indiscriminate clustering.

There are several duplications out of the 28 accessions collected. For example, RNR-10 from Babajipetha and RNR-25 from Timbaktu were the duplicates. Similarly, RNR-1 from Buditi and RNR-12 from Chipurupalli were also the duplicates. For landraces, the genetic variability is maintained not only between but also within the

accessions. The molecular markers, along with the morphological traits, made it possible to evaluate the genetic diversity contained within and between the cultivars and also helped in identifying the duplicate accessions in the gene banks (Virk *et al.*, 1996; Zhu, 1996). However, there is relatively little information available on the intra-accession (cultivar) variation in landraces compared with the variation between them. It is important that the agro-morphological characterization be further explored by more sophisticated studies, both biochemically and molecularly to test the validity of our results. The identification of duplication will lead to a good ex-situ conservation strategy of the accessions. The high duplication rate shows the importance of the exchange of the seeds between the producers within a village or at the state level. The occurrence of duplicates, evident in the dendrogram, indicated the existence of a movement of the seeds within and between localities. Markets remain the main exchange place for the seeds. The enhancement of the number of accessions of the same species and the use of different modes of characterizations can contribute to the implementation of a core collection, which is the best strategy of conserving this landrace germplasm.

4.3. Genetic Variability of Landraces

The objective of the present study is to estimate the amount of the genetic variability available for selection in the accessions and to estimate the amount of heritability and genetic advance among agro-economic traits. The amount of genetic variability is a major determiner of the genetic gain from selection. The estimates of the phenotypic variances were higher than their respective genotypic ones for almost all the traits under study except for the harvest index. In general, the agronomic characters had larger phenotypic variances than their respective genotypic ones. However, the influence of the environmental factors on the expression of the agronomic characters, as indicated by the magnitude of the phenotypic variance, was quite evident. This indicates that a large proportion of phenotypic variance was due to environmental causes; thus, such characters do not possess a promising genetic variation. For them, therefore, selection will not be effective; solely, it would prove to be very low.

The degree of variability, shown by different parameters, can be judged by the magnitude of GCV and PCV. In general, there were differences in the magnitude of genotypic and phenotypic coefficients of variation for all the traits under study. The PCV showed that the extent of the genetic variability in the population ranged from 11.18 (harvest index) to 79.87 (stalk yield). Presence of a high variability for stalk yield, total biomass and leaf yield, as evident from their GCV and PCV values for the above parameters, can form the basis for the effective selection of superior lines in Roselle. The PCV was slightly higher than the corresponding GCV for all the characters under study, which indicates the environmental influence on the character expression. For these characters, the differences between PCV and GCV were narrow, and the PCV and GCV values were close to one another, indicating a low environmental influence in the expression of these characters, implying that the genotype

contributed more in the expression of these characters than the environment, suggesting greater possibilities of improvement through selection. With the help of GCV alone, it is not possible to determine the extent of heritable variation. Thus, the estimates for the heritability indicate the effectiveness with which the selection may be expected to exploit the existing genetic variability.

The high magnitude (>60%) of the estimates of the heritability for plant height, stalk yield, leaf yield, total biomass per plant and leaf stalk ratio indicates that these characters possessed a wide range of genetic variability and their improvement could be achieved by mass selection alone. These high estimates of heritability could be attributed to the difficulty of the separation of all the genotype and environment interactions from genotypic variance since the study was carried out in one location and during one season, and, thus, the heritability estimates were biased upward. The moderate heritability for the harvest index was due to the fact that it depends on many components which are greatly influenced by the environment. Falconer (1980) opined that more variable conditions reduce the heritability, whereas uniform conditions increase it. The high heritability for plant height was also reported by Ibrahim and Hussein (2006) in Roselle. In a selection program where the primary objective is character improvement, a study of genetic gain is more advantageous than the heritability studies.

The high estimates of the heritability (>60%), coupled with a high genetic advance as percent of mean (>20%), were observed for almost all the traits except for the harvest index (Table 5). The high heritability, coupled with a high genetic gain and coefficient of variability, was observed for fresh sepal's weight, number of capsules and plant height (Sanoussi *et al.*, 2011; Falusi *et al.*, 2014). High estimates of heritability (>60%), coupled with a high genetic advance as percent of mean (>20%) for plant height, total biomass, leaf yield, stalk yield and leaf-stalk ratio, indicate the possibility to improve these agro-economic traits through selection programs to develop new varieties. However, the association of the genetic advance and heritability does not follow the same pattern as that between the genetic advance and the genotypic coefficient of variation. The increase in the heritability value was not always accompanied with an increase in the genetic advance. The nature of association between the heritability and the genetic advance was explained by Panse (1957) who reported that the association of high heritability with a high genetic advance is an indication of additive gene effects and, consequently, a high genetic gain from the selection could be expected. On the other hand, the association of low heritability with a low genetic advance is an indication of non-additive gene effects and, consequently, a low genetic gain would be expected from the selection. However, heritability does not provide an actual measurement of the amount of the genetic variation, as the magnitude of the heritability depends on the degree of the association between the genotypic and the phenotypic variances regardless of being high or low; while, the genetic gain depends on the amount of the genetic variability (Johnson *et al.*, 1955). A similar line of reasoning was expressed by Allard (1999).

5. Conclusion

In conclusion, the analysis of variance revealed that the mean squares, due to genotypes, were significantly different, indicating significant differences in the landraces for all the six agro-economic traits under study. The landraces RNR-16 (14.22 g/plant), RNR-20 (12.72 g/plant), RNR-27 (11.85 g/plant) were of a high leaf yielding potential and, thus, are highly suitable to grow as monthly crops. The multivariate analysis, following Ward's minimum variance method, revealed a distinct clustering pattern of landrace germplasm. The distinct clustering pattern, as evident from the dendrogram, presumably reflects the divergence of landraces. Using more landrace accessions and other evaluation methods could help set up a core collection, which is the best way for germplasm conservation. This Roselle landrace germplasm was endowed with a rich genetic variability for all agro-economic traits, as evident from the genotypic and phenotypic coefficients of variation. The broad sense heritability and the genetic advance indicated that the selection for plant height, leaf yield, stalk yield, leaf-stalk ratio and total biomass would be more effective in boosting the leaf yield performance of the vegetable Roselle genotypes. Selection is effective for plant height, leaf yield, stalk yield, leaf-stalk ratio and the total biomass in Roselle.

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