VARIABILITY STUDIES BASED ON MORPHOLOGICAL AND BIO-CHEMICAL ANALYSIS IN RUMEX (*Rumex spp.*) ACCESSIONS

by

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(J-22-MHF-21)

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for the degree of

MASTER OF SCIENCE IN HORTICULTURE
(VEGETABLE SCIENCE)

Division of Vegetable Science
Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu,
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2024

CERTIFICATE - I

This is to certify that the thesis entitled, **"Variability studies based on morphological and bio-chemical analysis in Rumex *(Rumex spp.)* accessions"** submitted in partial fulfillment of the requirements for the degree of **Master of Science in Horticulture (Vegetable Science)** to the Faculty of Horticulture and Forestry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, is original work and has similarities with published work not more than minor similarities as per UGC norms of 2018 adopted by the University. Further, the level of minor similarities has been declared after checking the manuscript with **DRILLBIT** software provided by the University.

The work has been carried out by **Mr. Sunit Jarngal,** Registration No. **J–22–MHF-21** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma. It is further certified that help and assistance received during the course of thesis investigation have been duly acknowledged.

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Sunit Jarngal

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ABSTRACT

Title of Thesis	:	**"Variability studies based on morphological and bio-chemical analysis in Rumex (*Rumex spp*) accessions"**
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The research entitled, **"Variability studies based on morphological and bio-chemical analysis in Rumex (*Rumex spp.*) accessions"** was carried out during the rabi season 2023 at Vegetable Experimental Farm of SKUAST-Jammu to study variability and genetic diversity among the accessions of *Rumex* on the basis of morpho-metrical and bio-chemical characters. The experiment was laid out in Randomized Complete Block Design consisting of 25 accessions with three replications. The analysis of variance revealed the existence of a substantial amount of variability in the germplasm. On the evaluation of mean performance, two accessions namely SJRU-33 and SJRU-12 performed statistically better than all other accessions for most of the yield and yield contributing traits, whereas, accessions namely SJRU-12, SJRU-72, SJRU-33, SJRU-24 and SJRU-61 performed statistically better than all other accessions for most of the bio-chemical characters. This signifies their role in the nutrition, pharmacological and cosmetics industries. They are also only utilised for cancer prevention, immune support, gut and eye health etc. and possess natural coloring properties with high energy potential. In general, estimates of phenotypic coefficient of variance (PCV) was found higher in magnitude than corresponding genotypic coefficient of variance (GCV) estimates. The phenotypic and genotypic coefficient of variability were found to be high for green leaf yield per plot and per hectare. The number of leaves per plant and green leaf yield per hectare showed high heritability coupled with high genetic advance as percentage of mean, suggesting additive gene action that can be improved through direct selection. The green leaf yield per plot and per hectare had positive and significant correlation with number of pickings per plant and number of leaves per plant, whereas, total phenolics had positive and significant correlation with total flavonoids, DPPH free radical scavenging, total sugars and total carotenoids. Thus, selection may be possible for these characters for improving yield and quality characters respectively. The maximum positive direct effect towards yield per hectare was contributed by number of leaves per plant followed by number of pickings per plant and leaf length. Based on D^2 statistics, 25 accessions of *Rumex* were grouped into seven and five clusters separately for morpho-metrical and bio-chemical characters respectively. The maximum inter cluster distance was observed between cluster III and cluster VI for morpho-metrical traits and between cluster II and cluster IV for bio-chemical characters. Hence, the accessions belonging to these divergent clusters can be used in hybridization programme.

Keywords: *Rumex spp.* L., genetic diversity, heritability, genetic advance, morpho-metrical, bio-chemical, path coefficient, variability

Signature of Major Advisor

Signature of the Student

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ABBREVIATIONS

%	:	Per cent
°C	:	Degree Celsius
°N	:	Degree North
ANOVA	:	Analysis of variance
AOA	:	Antioxidant activity
cm	:	Centimeter
CV	:	Coefficient of variation
DPPH	:	2,2-diphenyl-1-picrylhydrazyl
df	:	Degree of freedom
et al.	:	Et alia meaning ‘and others’
g	:	Gram
GAE	:	Gallic acid equivalent
GAM	:	Genetic Advance as percentage of mean
GCV	:	Genotypic coefficient of variation
Kg	:	kilogram
h^2 (bs)	:	Broad sense heritability
mg	:	Milli gram
mL	:	Milli liter
mm	:	Millimeter
N	:	Normality
PCV	:	Phenotypic coefficient of variation
QE	:	Quercetin equivalents
SD	:	Standard deviation
UV	:	Ultraviolet
viz.	:	Videlicet meaning ‘namely’
cm^2	:	Square centimeters

$\sum$	:	Summation
nm	:	Nanometer
λ	:	Lambda
χ^2	:	Chi-square
rg	:	Genotypic correlation
rp	:	Phenotypic correlation
μL	:	Microliter
SE	:	Standard Error of the mean
$CD_{0.05}$	:	Critical difference at 5 per cent
D^2	:	D square

CHAPTER-I

INTRODUCTION

Rumex spp. (2n=2x=14) or Sorrel is an under-utilized vegetable crop that belongs to family *Polygonaceae* and comprises of about 50 genera and 1200 species. Important genera include *Rheum*, *Rumex*, *Polygonum*, *Coccoloba*, *Calligonum* and *Persicaria* etc. The name rumex was adopted by Linnaeus (1753) from ancient Latin word "Rue-mex" (Dock and Sorrel) and its phylogeny with basic chromosome number x=7 was reported by Navajas-Perez *et al*., 2005. The genus comprising about 200 species, is the second largest assemblage of the family Polygonaceae (Airy-Shaw, 1973). Some species of rumex are worldwide in distribution, varying in habitat from wet soils and cultivated fields to the xerophytic climates of sandy regions. Plants of the rumex genus are common in Europe, Asia, Africa, and North America, but more widely spread in the temperate zone of the northern hemisphere. In the Indian sub-continent, the genus Rumex is represented by 13 species (Santapau and Henry, 1973), of which 9 are found in Jammu and Kashmir (Stewart, 1972).

Rumex are herbs, rarely shrubs, usually with long, stout roots, sometimes rhizomatous. Leaves are alternate, ochreae tubular, basal leaves are acute and narrowly lanceolate to oblanceolate. Petioles are canaliculate above and their inflorescence is dense.

Rumex contains carbohydrates (3.2 g), fat (0.7 g), protein (2g), thiamine (0.04 mg), riboflavin (0.1 mg), niacin (0.5 mg), calcium (44 mg), iron (2.4 mg), magnesium (103 mg), phosphorus (63 mg), potassium (390 mg) and zinc (0.2 mg) per 100g of dry matter (Stephen and Suresh, 2015). The medicinal importance of this plant is a reflection to its chemical composition since the plant contains many bioactive substances such as flavonoids (vitexin, isovitexin, orientin and isorientin), anthraquinones (emodin and chrysophanol), carotenoids, vitamins especially vitamin C, proteins, lipids and organic acids (Mostafa *et al*., 2011). The bioactive phytochemicals (such as polyphenols, flavonoids, carotenoids, tocopherols and ascorbic acid) have a role as antioxidant and detoxifying agents. Their intake leads to the protection against non-communicable diseases in human beings such as cancer, cardiovascular diseases and cataract (Rao *et al.,* 2003; Matkowski *et al.,* 2008).

Rumex leaves are a good source of macronutrients and micronutrients, and it has been suggested to use them as an economic source of plant-based protein (Ladeji and Okoye, 1993). However, the presence of high levels of the anti-nutritive factor like oxalic acid has been reported by some workers, thus causing mineral deficiencies and other serious problems such

as calcium oxalate stone formation in kidneys and decreased iron absorption (Korpelainen and Pietilainen, 2020). This plant is a good source of minerals like K, Na, Ca, Mg, Fe, Mn and Cu. Usually rumex plants has anti-inflammatory (Humeera *et al*., 2013; Vasas *et al*., 2015), bactericidal (Lee and Rhee, 2013), anti-dermatitis, astringent and antitumor (Litvinenko and MuzychKina, 2003) properties.

There have been numerous ethnobotanical and ethnopharmacological reports dealing with the occurrence and traditional uses of *Rumex species*. It is commonly combined with other greens and eaten raw or cooked in soups, stews and pastries. Rumex is widely mentioned in gardening and recipe books because of its distinctive sour taste, which is attributed to its high oxalic acid content. The tangy, acidic, sour-lemony flavor of sorrel blends well with a variety of ingredients, such as meat, cheese and milk. Sorrel sprouts and young immature leaves have a more subtle flavour and so are suitable for addition to salads and sandwiches. The more mature leaves are normally cooked and added to soups and stews and used to make sauces.

The use of rumex as a vegetable, that provides a nice citrusy flavor to a variety of dishes has been proven (Jarić *et al.,* 2007; Mattalia *et al.,* 2013; Pieroni *et al.,* 2014). *Rumex spp.* are used as vegetables, eaten fresh or cooked, by the locals in Kashmir; however, in Jammu plains, it is grown wild in all fields but its importance is still unknown to the large populace. Keeping in view the importance and diversity of this crop, the present research work entitled "**Variability studies based on morphological and bio-chemical analysis in Rumex (*Rumex spp.*) accessions**" has been conducted with following objectives:

Objectives

1. To evaluate variability among the accessions of Rumex on the basis of morphological and bio-chemical traits.
2. To study genetic diversity and association among accessions of Rumex species.

CHAPTER-II

REVIEW OF LITERATURE

The Polygonaceae are a family of flowering plants such as rumex (*Rumex species*), rheum (*Rheum rhabarbarum*), buckwheat (*Fagopyrum species*), smart weed (*Persicaria species*), etc. The literature relevant to present investigation is compiled to present an insight of the diversity and importance of *Rumex species* and related species (*Fagopyrum*, *Rheum* and *Amaranthus*) under following heads:

2.1 Morpho-metrical parameters

Zhang *et al*. (2004) analysed the genetic polymorphisms of three continued generations of Rumex k-1 (*R. patientia* × *R. tianschanicus* cv. Rumex k-1) and several wild *Rumex spp.* using random amplified polymorphic DNA markers (RAPD). The result showed that there were no different polymorphic loci among the three successive generations of Rumex k-1, showing genetic stability, except for 12 plants of the certified seed of Rumex k-1; while among the different populations of the same wild Rumex species growing in different sites, there appear abundant genetic polymorphisms loci.

Zabinska *et al*. (2005) examined genetic diversity of 6 populations of *Rumex confertus* localized in different parts of Poland. The research reported relatively low level of inter and intra population genetic diversity of analyzed populations.

Kapila *et al.* (2006) characterized 47 genotypes of buckwheat collected from higher Indian Himalayas and evaluated for 6 important agro-morphological traits. The data exhibited presence of sufficient variability. Agro-morphological traits like plant height, primary branches/plant, days to maturity and 100-seed weight were found important in distinguishing buckwheat genotypes. The estimates of PCV and GCV were lower for days to maturity, days to flowering and plant height as compared to remaining 3 traits which exhibited wider range of variation in the present material. Cluster analysis grouped genotypes in 13 clusters with a maximum of 20 genotypes in cluster I, followed by 13 in cluster II, 3 in cluster XIII, 2 in cluster X and others remaining ungrouped.

Raycheva *et al*. (2013) sampled 12 accessions each of *R. patientia*, *R. cristatus*, *R. confertus*, and *R. alpinus* with *R. pulcher* as a referent species for the assessment of their genetic diversity using ISSR markers. The combination of the results from the used primers classified the studied species into two major groups: Group 1 with *R. patientia*, *R. cristatus* and *R.*

confertus with genetically similar ploidy level; Group 2 includes the diploid *R. alpinus* and the referent species *R. pulcher*.

Bisht (2015) evaluated 30 diverse genotypes of buckwheat including four checks i.e. PRB-1, Himpriya, VL-7 and Shimla-B1. The cultivars PRB-1 and IC-13533 exhibiting high mean performance for seed yield along with high performance for some other yield components. High phenotypic and genotypic coefficient of variation (PCV and GCV), heritability and genetic advance were observed for plant height, days to 50% flowering, days to maturity, number of leaves per plant, 100-seed weight and seed yield per plant. Correlation studies indicated that seed yield per plant was positively correlated with days to maturity, plant height and number of primary branches per plant. The genotypes were grouped into 6 clusters having maximum inter-cluster genetic distance between cluster I and cluster IV followed by cluster I and cluster III suggesting wide diversity among these groups.

Munir *et al*. (2016) conducted a study to access the genetic variability and association among morphological traits of *Rumex dentatus* under four different locations showing significant differences. Results of morphological traits such as plant height (22.179±1.2011cm), leaf area (28.948±0.1082cm^2), fresh inflorescence weight (4.074±1.0121g), dry plant weight (0.588±0.0047g) and moisture content percentage (88.483±2.67%). The coefficient of variation was found to be lower for all studied traits. The results indicated that the fresh plant weight was significantly related with dry weight and moisture content percentage in the plants. Hence, it was suggested that the growth and development of *Rumex dentatus* should be controlled through proper techniques to reduce yield losses in crop plants.

Hiremath *et al*. (2017) evaluated 54 diverse buckwheat genotypes (involving exotic, indigenous and local collection) at University of Agricultural Sciences, Dharwad, Karnataka to estimate the phenotypic coefficient of variation (PCV), genetic coefficient of variation (GCV), heritability and genetic advance as per cent mean, in a replicated CRBD during Kharif season. Analysis of variance revealed highly significant differences between the genotypes for all characters studied suggesting the presence of adequate variability for selection. Moderate PCV and GCV coupled with high heritability were recorded for days to 50 per cent flowering while, high genotypic and phenotypic coefficients of variation coupled with high heritability and genetic advance as per cent mean were observed for seed yield and number of secondary branches indicating the more additive gene action hence the selection for these traits could be

effective. However, days to maturity exhibited low PCV, GCV and genetic advance indicating selection will be ineffective for such traits.

Bisht *et al.* (2018) estimated the genetic variability, correlation and path coefficient analysis of yield and yield contributing traits in 30 diverse germplasm line of buckwheat including four check varieties PRB-1, Himpriya, VL-7 and Shimla-B1 at V.C.S.G. Uttarakhand University of Horticulture and Forestry, College of Forestry, Ranichauri, Tehri Garhwal, Uttarakhand. The analysis of variance indicated that the treatments were highly significant for all the traits. Genotypic and phenotypic variance was high in plant height ensued by days to 50% flowering. The maximum heritability in broad sense was realized for days to 50% flowering and high value of genetic advance mean recorded for seed yield per plant. The genotypic correlation was generally similar in nature and higher in magnitude than corresponding phenotypic correlation coefficient. Path coefficient analysis indicated that days to maturity, plant height, number of primary branches per plant and 100-seed weight showed positive direct effect to seed yield per plant. It is suggested that these characters can be considered as selection criteria in improving the seed yield of buckwheat germplasm.

Misra *et al.* (2019) characterized 97 accessions of *Fagopyrum esculentum* and *F. tataricum* for 16 morphological descriptors. Significant variations were observed for agronomically important traits including days to 50% flowering, days to 80% maturity, primary branches plant, plant height and 100-seed weight.

Sharma (2022) evaluated 43 genotypes of buck wheat including three checks for the nature and extent of genetic variability and associations (direct and indirect effects) among various traits. Analysis of variance revealed that there is adequate genetic variation for all traits under study. High genotypic and phenotypic coefficients of variation was observed for seed yield per plant. Moderate GCV and PCV was observed for plant height, number of primary branches per plant and 100-seed weight. While low GCV and PCV was observed for days to 50 % flowering, days to 80 % maturity, plant stand at harvest, crude protein and harvest index. High heritability coupled with high genetic advance was observed for seed yield per plant. High heritability with moderate genetic advance was observed for plant height, the number of primary branches per plant and 100-seed weight whereas, High heritability coupled with low genetic advance was observed for days to 50 % flowering, days to 80 % maturity, plant stand at harvest, crude protein and harvest index. Correlation analysis revealed that seed yield per plant had a significant positive correlation with days to 80 % maturity, plant stand at harvest, plant height, number of primary branches per plant, 100-seed weight and harvest index. Path

analysis revealed that direct positive effects of plant stand at harvest followed by plant height, 100-seed weight and harvest index towards seed yield per plant hence, these traits can be considered for direct selection for high seed yield. Mahalanobis D2 statistics grouped 43 tartary buckwheat genotypes into five clusters. Cluster I and cluster II contained seven and 37 genotypes respectively and the remaining clusters viz., III, IV and V were solitary, each containing a single genotype.

Chang *et al*. (2023) investigated the genetic diversity of *Rumex patientia* L. in distinct habitat types. The results showed that the inter-population variation accounted for 36.62% of the total variation and the intra-population variation accounted for 63.38%. Cluster analysis based on Nei's genetic distance showed that the greatest genetic distance between E and S (0.1218) and the closest genetic distance between W and N (0.0794).

Jungova *et al*. (2023) studied rumex species to verify whether *R. alpinus* was introduced into the Krkonose mountains by alpine colonists or whether it was anthropogenically introduced from the Carpathians. Furthermore, the genetic structure of native and introduced populations of *R. alpinus* was determined. The results of AMOVA showed a high 60% variation within populations, 27% variation among groups, and 13% among the population within groups. The overall unbiased gene diversity was high ($\hat{h}$= 0.55). The higher level of genetic differentiation among populations (FST = 0.35; $p<.05$) indicated restricted gene flow between populations. Compared to native populations, limited genetic variability was observed in the non-native populations. It was concluded that local adaptation, low gene exchange, and genetic drift affected the genetic diversity of non-native *R. alpinus*.

Papaiahgari *et al.* (2023) analysed the correlation and path analysis in 40 buckwheat genotypes during Rabi, 2021-2022 at the Department of Genetics and Plant Breeding, SHIATS, Allahabad in Randomized Block Design. Analysis of variance showed significant difference for seed yield and its components indicating presence of large amount of variability in the genotypes. The magnitude of GCV and PCV recorded highest for economic yield, plant height, time of beginning of flowering, number of branches, test weight, days to 50% flowering. High heritability coupled with high genetic advance as percent mean was recorded for economic yield, plant height, time of beginning of flowering, test weight, number of branches, days to 50% flowering, days to 80% maturity. Seed yield per plant showed a highly significant positive correlation with test weight and days to 80% maturity at both the genotypic and phenotypic levels. Path analysis revealed that test weight and time of beginning of flowering registered high and positive direct effect on seed yield per plant. It suggested that there is a true

relationship between these traits, and that direct selection for these traits will be beneficial for yield improvement.

Singh *et al.* (2024) analyzed 24 accessions of tartary buckwheat with some local germplasm by cluster and principal component using 17 quantitative characters to group them on the similarity at phenotypic level, and to identify the most relevant characters in explaining the variation. The genotypic variance of all the traits was lower than the phenotypic variance. Highest GCV and PCV were recorded for seed yield per plant, seed per plant and protein content. The non-hierarchical Euclidean cluster analysis using Mahalanobis statistic, grouped the genotypes into five distinct clusters among which two clusters were polygenotypic and three clusters were monogenotypic. Cluster I was the largest group comprising of 15 genotypes characterized with highest primary branch per plant, inflorescence length, leaf per plant, seed per plant, seed yield per plant, magnesium content, iron content and zinc content. Most of the high yielding germplasm along with Himpriya were grouped under this cluster. Cluster II composed of the genotypes having high amount of protein, calcium and phosphorus that can be used as a source for introgression of genes responsible for high nutrition.

2.2 Bio-chemical parameters

Yıldırım *et al.* (2001) studied the antioxidant activities, reducing powers, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activities, amount of total phenolic compounds and antimicrobial activities of ether, ethanol and hot water extracts of the leaves and seeds of *Rumex crispus* L.. The antioxidant activities of extracts increase with increasing amount of extracts (50-150 µg). However, the water extracts of both the leaves and seeds have shown the highest antioxidant activities. Among all of the extracts, the highest amount of total phenolic compound was found in the ethanol extract of seeds, whereas the lowest amount was found in the ether extract of seeds. Like phenolic compounds, the highest reducing power and the highest DPPH scavenging activity were found in the ethanol extract of seeds. However, the reducing activity of the ethanol extract of seeds was ~40% that of ascorbic acid, whereas in the presence of 400 µg of water and ethanol extracts of seeds scavenging activities were about 85 and 90%, respectively. There were statistically significant correlations between amount of phenolic compounds and reducing power and between amount of phenolic compounds and percent DPPH scavenging activities (r) 0.99, $P < 0.01$, and (r) 0.864, $P < 0.05$, respectively and also between reducing powers and percent DPPH scavenging activities (r) 0.892, $P < 0.05$.

Coruh *et al*. (2008) evaluated total phenolic content, antioxidant activity and antibacterial activity of aqueous and methanol extracts of the aerial part of the *Rumex crispus* L. naturally grown in the Eastern Anatolia region of Turkey. The aerial part of the plant had high total phenolic content (56.31 µ g/mg DW). The antioxidant activity of aqueous and methanol extracts of the aerial part (BHA and BHT) were found to be 92.35%, 95.49%, 98.16%, and 96.66% respectively. Although the antioxidant activity of the aqueous and methanol extracts of *R. crispus* was lower than that of the BHA and BHT, the difference between these was not statistically significant, $p < 0.05$. The methanol extract possessed strong antibacterial activity against *Agrobacterium tumefaciens, Bacillus cereus, Bacillus subtilis, Pseudomonas corrugate, Pseudomonas syringae* pv. *tomato, Salmonella typhimurium, Serratia liquefaciens, Vibrio cholerae, Yersinia frederiksenii* and *Yersinia pseudotuberculosis*. Therefore, the aerial part of *R. crispus* can be used as an effective and safe source of antioxidants and antibacterial agent.

Sokol-Letowska *et al.* (2009) investigated the antioxidant activity of *Rheum palmatum* extracts. Antiradical activity against DPPH and ABTS radicals, reducing power FRAP and total phenolic contents, were investigated in one-, two- and three-year-old roots of rhubarb fertilized with nitrogen at the rates of 50, 100 and 200 kg N/ha. It was proved that nitrogen dose as well as the age of plantation did significantly influence antioxidant activity and total phenolic of root extracts. The highest values were determined in one-year-old plants, antioxidant activity ranged the level of 112–203 µM Trolox/g and total phenolic compounds average content was 22 mg GAE/g FW. When nitrogen dose increased, polyphenols content, DPPH and FRAP values increased as well, although, ABTS showed a different tendency.

Harshaw *et al*. (2010) investigated the bioactivities of n-hexane, dichloromethane (DCM) and methanol (MeOH) extracts of the leaves of *Rumex obtusifolius* were assessed using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay, the newly developed micro-titer-based antimicrobial assay incorporating resazurin as an indicator of cell growth, and the brine shrimp lethality assay. The most potent free radical scavenging activity was displayed by the MeOH extract with a RC50 value of 7.80 x 10-2 mg/mL. Among the fractions obtained from solid-phase extraction (SPE) of the MeOH extract, the 50% aqueous methanolic SPE fraction exhibited the highest levels of free radical scavenging property (RC50 = 1.05 x 10-2 mg/mL). While the n-hexane extract did not show any antibacterial activity at test concentrations, the DCM extract was active only against *Escherichia coli*. However, the MeOH extract as well as

the 50% and 80% SPE fractions of the MeOH extract showed significant antibacterial property against all bacterial strains tested.

Park *et al*. (2010) analyzed the ordinary ingredient and physical and chemical features of the *Rumex crispus*. The ordinary substance analysis of the *Rumex crispus* has shown that it contains 5.50～17.60%, of crude protein, 0.30～0.83% of crude fat, 5.66～12.14% of crude ash and 12.60～53.22% of crude fiber. The reducing sugar analysis showed that the leaves contain 3.34%, the stem 2.26%, the root 12.05% respectively. The total sugar analysis showed that the leaves contain 16.36%, the stem 5.74%, the root 67.19% respectively.

El-Hawary *et al*. (2011) investigated the bioactive compounds in extracts of *Rumex vesicarius* L. in Egypt and identified 13 phenolic compounds, out of which 6-C-glucosyl-naringenin was the major compound. The biochemical studies attributed various compounds referring to the antioxidant potential, membrane stabilizing effect and anti-fibrogenic activities of this crop.

Mostafa et al. (2011) evaluated antibacterial and antioxidant activities of different plant parts of *Rumex vesicarius* L.. Different extracts of various organs of *Rumex vesicarius* L. (leaves, stems, roots, flowers, whole plant parts and fruits), were screened for their antibacterial activity against six human pathogenic bacterial isolates by disk diffusion assay. Antioxidant activity was determined spectrophotometrically using total antioxidant activity and DPPH scavenging activity methods. Stems were found to have the highest total antioxidant capacity (17.458 GA equivalents "ppm"). Regarding DPPH scavenging activity, fruits were found to be the most effective plant parts (IC50 =0.731 mg/ml). Total phenolics were estimated, fruits extract was found to be the richest one in this regard (15.633 mg gaes/g F.W.). Total flavonoids were estimated, fruits extract was found to be the highest containing one in this regard (25.995 µg/g F.W.). HPLC analysis of flavonoids was carried out using Quercetin as standard, whole plant parts extract was found to contain the highest amount of Quercetin (82.452 µg/g D.W.).

Sahreen *et al.* (2011) studied different solvent fractions (n-hexane, ethyl acetate, chloroform, butanol and aqueous) of the methanol extract of *Rumex hastatus* (RH) leaves for antioxidant activities. The ethyl acetate fraction possessed high amount of total polyphenolics (106.3±3.93 mg GAE/g extract) while butanolic fraction was found with high flavonoid contents (59.8±1.94 mg RTE/g extract) as compared to other solvent fractions. Butanol fraction exhibited most promising DPPH (EC50 140±0.99 µg/ml), iron chelation (EC50 70±2.1 µg/ml) and for phosphomolybdate (EC50 31±1.25 µg/ml) scavenging activity. However, ethyl acetate

fraction exhibited the highest scavenging potential for super oxide radicals (EC50 40±0.78 µg/ml), ABTS radicals (EC50 70±2.46 µg/ml), hydroxyl radicals (EC50 9±0.56 µg/ml) and inhibition of β-carotene linoleic acid peroxidation (EC50 54±5.67 µg/ml). Chloroform fraction showed the most potent antioxidant activities in scavenging of hydrogen peroxide (EC50 48±4.89 µg/ml). The high correlation coefficient was found between EC50 values of DPPH, superoxides, hydroxyl, hydrogen peroxide and ABTS radicals with total polyphenolic and flavonoids.

El-Bakry *et al*. (2012) investigated some biologically active constituents and antioxidant activity of different plant parts of *Rumex vesicarius* L. at the vegetative stages of growth (early and late vegetative stages). Total phenolics in different plant parts showed that whole plant parts (at late vegetative stage) were the richest organ in this regard (4.518±0.018mg GAEs/g F.W.). It was found that all plant parts at early vegetative stage of growth were rich in flavonoids. Leaves contained the highest amount of flavonoids (31.11±0.25 µg/g F.W.). Regarding antioxidant activity studies, using total antioxidant activity and DPPH scavenging activity methods, it was found that root (at late vegetative stage) extract had the highest amount of total antioxidants (428606.000±4792.885 GAEs as ppm). Results of DPPH scavenging activity studies revealed that the least IC50 (the highest the effectiveness) was obtained using leaves (at early vegetative stage) extract (IC50= 0.345±0.005 mg/ml).

Elzaawely *et al.* (2012) investigated total phenolics, antioxidant capacities assayed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging and β-carotene bleaching methods and reducing power in different extracts/fractions of leaves and roots of *R. dentatus* grown in Egypt. In addition, their phenolic compositions were determined by GC-MS and HPLC. The results showed that total phenolic content in the ethyl acetate fractions of leaves and roots were high and measured at 169.5 and 257.4 mg gallic acid equivalent per g extract, respectively. The ethyl acetate fractions of leaves and roots exhibited strong DPPH activity and the DPPH IC50 values were 0.021 and 0.012 mg mL^{-1} of leaves and roots, respectively. Furthermore, the ethyl acetate fractions of leaves and roots showed high reducing power and antioxidant activity assayed by β-carotene bleaching method. GC-MS and HPLC analyses indicated that these fractions contained a variety of phenolic compounds.

Foda *et al*. (2013) examined phenolic compounds in *Rumex vesicarius* L. by both liquid chromatography/mass spectrometry (LC/MS) and by gas chromatography/mass spectrometry (GC/MS). Results showed that extract of *Rumex vesicarius* had high amount of phenolic

compounds. The results of antibacterial activity and total phenolics of *R. vesicarius* L. exihibit strong association which reflects the antibacterial activity and antioxidant capacity of the plant.

Kilic *et al*. (2013) evaluated the antioxidant activity of water extract of R. *conglomeratus* using different antioxidant tests. In addition, carotenoid content and proline level of *R. conglomeratus* were also determined. The results were compared with natural and synthetic antioxidants. The results showed that *R. conglomeratus* contained naturally occuring antioxidant components and possessed antioxidant activity which may be attributed to its radical scavenging and metal chelating activities. It was concluded that *R. conglomeratus* might be a potential source of antioxidants.

Duma *et al.* (2014) investigated chemical composition of three rhubarb cultivars of *Rheum spp*. – 'Victoria', 'Ogre', 'Tukums' grown in open field area during vegetation. It was clarified that content of oxalate in petioles increased during all of the vegetation time of rhubarb. 'Tukums' had the highest average content of phenolics (112.19±8.91 mg gallic acid 100 g^{-1} FW), flavonoids (7.36±0.28 mg catechin 100 g^{-1} FW) and antioxidant activity (11.95±0.02 mM Trolox ekv 100 g^{-1} FW) comparing with cultivars 'Ogre' and 'Victoria'.

Khan *et al*. (2014) assessed *in-vitro* antioxidant activity of different fractions and perform high performance thin layer chromatography fingerprint analysis of most active fraction of *Rumex vesicarius* L. In the present study, acetone, ethyl acetate, n-butanol, and methanol extracts of *R. vesicarius* were evaluated for radical scavenging activity by studying the inhibition of the level of lipid peroxidation induced by Fe(++)/ascorbate, DNA sugar damage, scavenging of hydrogen peroxide, diphenylphosphine DPPH radical scavenging activity, total phenolic content, total flavonoids content and total proanthocyanidin. The methanolic extract showed the highest total phenolic, total flavonoids and total proanthocyanidin contents.

Beddou *et al.* (2015) evaluated the antioxidant activities of hydroalcoholic extract (HAE) and its fractions (viz., hexane (HF), chloroform (CF), ethyl acetate (AF), n-butanol (BF) and water (WF) obtained from aerial part of *Rumex vesicarius* L. by using different *in vitro* antioxidant assays. The content in pigments (carotenoids and chlorophylls), total phenolics, flavonoids and tannins were determined using spectrophotometric methods. Experimental results obtained show that *R. vesicarius* is a rich source of β-carotene (116.83±1.60 μg/g DW), lycopene (156.40±1.59 μg/g DW) and chlorophyll a (271.45± 3.46 μg/g DW). The greatest antioxidant activity was found in AF (IC50.DPPH=0.07±0.00 mg/ml) followed by BF and CF

(0.15±0.00 and 0.16±0.00 mg/ml, respectively). The amount of total phenolics varied from 0.37±0.01 to 43.28±0.28 mg GAE/g of dry weight, HAE had the higher content (43.28±0.28 mg GAE/g of DW). A negative correlation was found between phenolic compounds and the antioxidant efficiencies of the crude extract/fractions, suggesting that phenolic compounds are not the only contributors to the antioxidant activities of *Rumex vesicarius*.

Vasas *et al*. (2015) in order to identify the therapeutic potential of *Rumex* species, provided an overview of the current state of knowledge of local and traditional medical uses, chemical constituents, pharmacological activities, toxicity and safety of *Rumex* species. The research confirmed that some *Rumex* species have emerged as a good source of the traditional medicine for treatment of inflammation, cancer and different bacterial infections and provides new insights for further promising investigations on isolated compounds, especially quercetin 3-*O*-glucoside, emodin, nepodin, torachrysone, and trans-resveratrol to find novel therapeutics and aid drug discovery.

Ahmad *et al*. (2015) investigated *Rumex hastatus* D. Don crude methanolic extract, subsequent fractions, saponins and flavonoids for acetylcholinestrase, butyrylcholinestrase inhibition and diverse antioxidant activities to validate its folkloric uses in neurological disorders. Antioxidant potentials of Rh. Sp and Rh. Fl were evaluated using DPPH, H2O2 and ABTS free radical scavenging assays at 62.5, 125, 250, 500, 1000 µg/mL. All the test samples showed concentration dependent cholinesterase inhibition and radicals scavenging activity. In DPPH free radical scavenging assay, Rh. Sp and Rh. Fl showed comparable results with the positive control i.e., 63.34 ± 0.98 and 76.93 ± 1.13% scavenging at 1 mg/mL concentration (IC50 312 and 104 µg/mL) respectively. The percent ABTS radical scavenging potential exhibited by Rh. Sp and Rh. Fl (1000 µg/mL) were 82.58 ± 0.52 and 88.25 ± 0.67 with IC50 18 and 9 µg/mL respectively. Similarly, in H2O2 scavenging assay, the Rh. Sp and Rh. Fl exhibited IC50 175 and 275 µg/mL respectively.

Ahmad *et al*. (2016) investigated the isolation of essential oil and its antioxidant and anticholinesterase assays. The essential oil of *R. hastatus* was analyzed by GC-MS for the first time and evaluated for anticholinesterase and antioxidant assays. The antioxidant potential was determined by using DPPH and ABTS free radicals. The antioxidant assays against DPPH and ABTS free radicals also exhibited significant scavenging potential with IC50 values of 3.71 and 6.29 µg/ml respectively, while for ascorbic acid the IC50 value was <0.1 µg/ml against both free radicals. It concluded that *R. hastatus* is an effective source of essential oil's components having anticholinesterase and antioxidant potentials.

Laouini and Ouahrani (2017) investigated the polyphenols profiling (total phenolic content, flavonoids, and condensed tannins), antioxidant and antibacterial properties of ethanolic extracts of seed, flower, leaf and stem from *Rumex vesicarius* L. Results showed that all extracts possessed concentration-dependent antioxidant activity. The study concludes that *Rumex vesicarius* L. possess diverse therapeutic potentials that might be used as natural antioxidant and antibacterial.

Feduraev *et al*. (2019) investigated the accumulation of phenolic compounds and the antioxidant activity of extracts of various parts of *R. crispus* and *R. obtusifolius*, collected at the flowering stage and the fruiting stage. The samples were analyzed for total catechins content, total proanthocyanidins content, total phenolic content, and total antioxidant activity and ferric reducing antioxidant power (FRAP) assays). The antioxidant activity arranged in the following order: the generative part (flowers, seeds) > leaves > root > stem (for flowering and fruiting stages). It was found that parts of the root closer to the stem differed in higher activity.

Katoch *et al*. (2019) investigation involves the nutritional analysis of three commonly grown weeds viz., *R. hastatus, R. obtusifolius, R. nepalensis* of mid-Himalayan region for determining their forage value. Crude protein, ash content, reducing sugars and cell content in *Rumex* species varied from 22.05 to 31.15%, 7.40 to 18.13%, 18.62 to 31.51%, and 56.46 to 59.00%, respectively. The polyphenolic compounds i.e., total phenols, simple phenols and total tannins were in the range of 3.43 to 7.46%, 1.40 to 3.35% and 2.03 to 4.11% respectively.

Sganzerla *et al*. (2019) evaluated the nutritional composition, bioactive compounds and antioxidant activity of *Rumex obtusifolius*. Results showed that hydroethanolic extract from root presented more bioactive compounds (p0.05) by DPPH assay than leaf extract. Pearson's correlation coefficients proved that antioxidant capacity is related to the presence of bioactive compounds in the root and leaf of *Rumex obtusifolius*.

Thakur and Baishya (2020) evaluated 16 buckwheat germplasm for biochemical constituents of quality significance. Total phenolic content was found between 378.41 to 652.71 mg/100g and flavonoids between 33.80 to 60.11 mg/100g on dry weight basis. The local genotypes were found comparable with the released genotypes in their nutrient composition and BWC-1, BWC- 2 and Kharupetia-2 along with buckwheat accessions EC-218742 and EC-27242 were found superior over others in terms of nutritional quality.

Kengne *et al*. (2021) studied the antibacterial, antifungal and antioxidant activities of extracts and compounds from *Rumex abyssinicus* Jacq. Antioxidant activity was evaluated

using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and gallic acid equivalent antioxidant capacity (GAEAC) assays. Compounds 1, 2 and the combined mixture of 6+7 displayed the highest antioxidant activity (GAEAC = 83.38–106.03 μg/mL).

Feduraev *et al*. (2022) analyzed seven species of the genus *Rumex* (*R. acetosa*, *R. acetosella*, *R. confertus*, *R. crispus*, *R. maritimus*, *R. obtusifolius* and *R. sanguineus* for subsequent extraction and analysis of phenolic compounds, as well as antioxidant activity. *R. acetosella*, *R. crispus*, *R. maritimus*, *R. obtusifolius*, and *R. sanguineus* were characterized by a high total content of phenolic compounds (111–131 mg g^{-1}). The maximum content of flavonoids was found in the leaves of *R. maritimus* and *R. acetosella*. Correlation analysis carried out during this study proved a positive relationship between antioxidant activity and the total content of phenolic compounds ($r = 0.785–0.921$, $p \leq 0.05$), flavonoids ($r = 0.602–0.918$, $p \leq 0.05$), proanthocyanidins ($r = 0.721–0.842$, $p \leq 0.05$), and tannins ($r = 0.591–0.776$, $p \leq 0.05$).

Hafaz *et al.* (2022) investigated 3 *Rumex* species collected from different habitats and tested them for antioxidant activity as well as for anti-microbial activity against some human pathogenic bacteria. The study indicated that the quantitative analysis of shoot and root extracts were found to be rich in tannins and phenolics composition. The aerial parts exhibited highest significant values compared to the root parts. The MeOH extracts of showed adequate antioxidant activity, wherein the IC50 values of the MeOH from the cultivated sample was 41.61 and 31.31 mg mL^{-1} , coastal samples were 34.99 and 23.99 mg mL^{-1} , while the sample of inland showed IC50 value of 41.59 and 31.67 mg mL^{-1} , for root and shoot, respectively.

Salama *et al*. (2022) assessed the biological potency and chemical composition of above ground parts of *Rumex vesicarius* using GC–MS. The major components were classified as fatty acids and lipids (51.36%), oxygenated hydrocarbons (33.59%), amines (7.35%), carbohydrates (6.06%), steroids (1.21%), and alkaloids (0.42%). The DPPH antioxidant activity of the extracted components of *R. vesicarius* verified that the shoot extract was the most potent with IC50 = 28.89 mg/L, with the percentages of radical scavenging activity at 74.28% ± 3.51%.

Bektasevic *et al*. (2022) overviewed *Rumex crispus* L. and *Rumex obtusifolius* L. in terms of their nutritional value as well as antioxidant and antimicrobial activity. The extracts of *R. crispus* and *R. obtusifolius* have been declared to possess antioxidant, antimicrobial, and antifungal activities, offering remarkable protection activity. The level of phenolic compounds

and other phytochemicals identified in these plants contributed to their radical scavenging activity and medicinal properties. Research to date indicates that *Rumex crispus* and *Rumex obtusifolius* can be potential sources of biologically active molecules intended for use in the pharmaceutical and food industry.

Alkaabi *et al*. (2023) explored the phytochemicals and proximate compositions from edible *Rumex vesicarius*. Extracts were prepared from the dried powder of the areal parts of the plant using methanol as solvent in Soxhlet. The antioxidant power of the extract was determined by using four different assays (ABTS, DPPH, superoxide anion and hydroxyl radical scavenging activity). In the concentration of total phenolic content (TPC), *R. vesicarius* showed a value of 38.48 in the aerial parts. The total flavonoid content in *R. vesicarius* was 10.24 in methanolic extracts.

2.3 Visual parameters

Wang *et al.* (2014) analysed the patterns of variation in leaf blade characteristics within and among populations through population-based sampling covering the entire distribution range of *Rheum palmatum* complex. Samples were taken from 2340 leaves from 780 individuals and 44 populations representing the four species, and the degree of leaf blade dissection and the shape of the lobe were measured to yield a set of quantitative data. The statistical analysis showed that the degree of leaf blade dissection is continuous from lobed to parted, and the shape of the lobe is also continuous from broadly triangular to lanceolate both within and between populations.

Thapa and Blair (2018) evaluated a germplasm collection of 260 amaranth accessions from United State Department of Agriculture (USDA) and 33 accessions from Seed Savers' Exchange (SSE). They evaluated morphological traits like blade pigmentation, blade shape, petiole pigmentation, branching index, flower color, stem color, inflorescence density, inflorescence shape, terminal inflorescence attitude, plant height and yield characteristics across all 293 accessions. Data analysis of morphological data showed significant difference of petiole pigmentation, stem color, blade pigmentation, blade shape and flower color across different clusters of accessions of USDA unlike among different clusters of SSE where we found significant difference of only blade pigmentation, blade shape and flower color.

Sonnberger (2023) studied neophytic populations of *Rumex longifolius* (Northern Dock) in the Alps and the mountain ranges bordering Bohemia. The populations differ in the length-width ratio of the basal leaf blades, the shape and size of the valves and the tendency to

develop tubercles of the valves. The morphological variability and ecological preferences of *R. longifolius* suggest its (experimentally yet not proved), hybrid origin from *R. aquaticus* and *R. crispus*.

CHAPTER-III

MATERIALS AND METHODS

The present investigation entitled "**Variability studies based on morphological and bio-chemical analysis in Rumex (*Rumex spp.*) accessions**" was carried out under sub-tropical conditions of Jammu. The details of the material used, specifics of the experimental and methodologies employed have been described as under:

3.1 EXPERIMENTAL SITE

3.1.1 Location

The Experimental Farm of the Division of Vegetable Science, Faculty of Horticulture and Forestry, Sher-e-Kashmir University of Agricultural Sciences and Technology, Main Campus, Chatha, Jammu (J&K) is situated at 32°40'N latitude and 74°58' E longitude at an elevation of 281.00 AMSL.

3.1.2 Climate

The climate of Vegetable Farm at Chatha is sub-tropical with a hot dry summer, hot humid rainy season, and cold winter months, the maximum temperature goes up to 42.4°C during summers (May to June) and the minimum temperature falls to 2.8°C during winters (Dec-Feb). The mean annual rainfall is about 1000-1200 mm. At the time of sowing, the maximum average weekly temperature went up to 23.8°C and the minimum weekly temperature fell to 7.2°C with relative humidity varying from 96-70% (morning to evening). During the vegetative phase, the maximum daily temperature ranged between 17.8-32.2°C and the minimum temperature fell between 2.5-18.6°C **(Source:** Agrometeorology observatory, SKUAST- J, Chatha). The meteorological data pertaining to the period of crop season in 2023-2024 is given in Appendix I.

3.1.3 Soil

The soil structure of the experimental farm is well-drained, fertile, loamy to clay loam rich in organic matter soils having pH ranging from 6.85 – 7.04.

3.2 EXPERIMENTAL MATERIAL

The experimental material comprised 25 diverse accessions of Rumex (*Rumex spp.*) collected from different areas of J&K. The details of the accession(s) are given in Table 3.1.

3.3 LAYOUT OF EXPERIMENT

3.3.1 Sowing

Seeds were sown in three replications on 5th Dec, 2023 in the Experimental Farm of the Division of Vegetable Science, SKUAST-Jammu.

3.3.2 Experimental design

The experiment was laid out in Randomized Complete Block Design and three replications with 25 plots (1.5m x 1.5m) in each block. The accessions were sown randomly in these plots to minimize biasness. The seeds were sown at the spacing of 40cm x 20cm in each plot.

3.3.3 Intercultural operations

First irrigation was given 3 days after sowing with rose cane and subsequent irrigation to keep the field under optimum moisture conditions was provided at 10 days interval.

3.3.4 Cultural practices

In addition to the application of farm yard manure (FYM) at the rate of 25 tonnes per hectare, chemical fertilizers (NPK) were applied according to the recommended guidelines provided for spinach beet by the Division of Vegetable Science, SKUAST-Jammu (Anonymous, 2022). The recommended dosage included 80 kg of nitrogen (N), 25 kg of phosphorus (P), and 25 kg of potassium (K) per hectare. The farm yard manure was mixed into the soil two weeks before sowing. Half of the prescribed N dosage and the full dosage of phosphorus and muriate of potash (MoP) were applied during plot preparation before sowing. The remaining N dosage was applied in splits after each first leaf picking followed by irrigation.

3.4 OBSERVATIONS RECORDED

All the observations were recorded as per standard procedures from five randomly selected plants of each accession in all three replications and their mean was worked out for statistical analysis. The characters studied and techniques adopted to record the observations are given below.

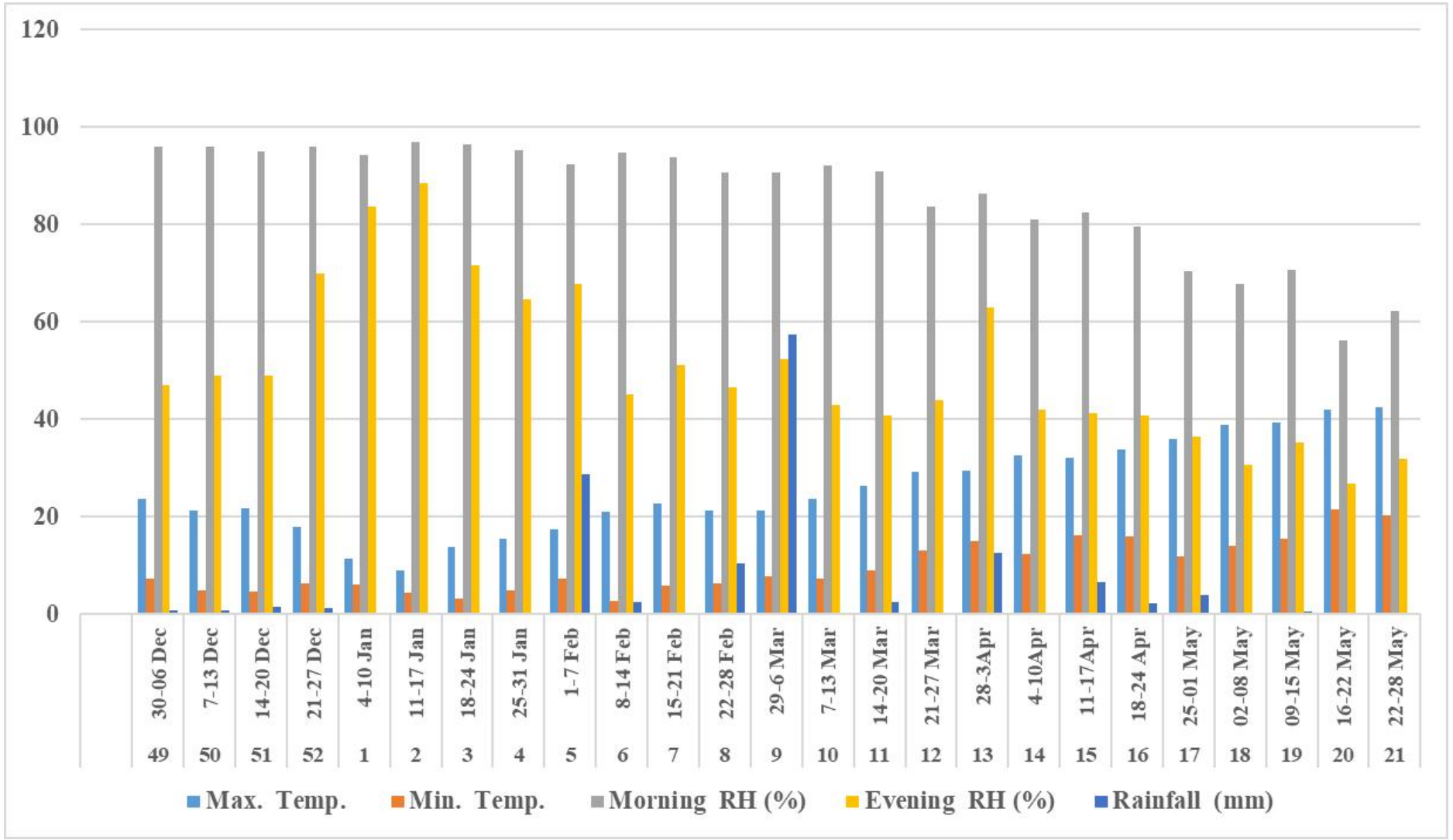


Figure 1: Graphical Representation of standard metrological weekly data for the year 2023-24.

Table 3.1 List of rumex accessions and their source of collection.

S. No.	Accession(s)	Source	Block	AMSL (metres)
1	SJRU-03	Hansa	RS Pora, Jammu	270
2	SJRU-04	Gharani	RS Pora, Jammu	270
3	SJRU-12	Tare chak	Bishnah, Jammu	292
4	SJRU-14	Dhinday Kalan	RS Pora, Jammu	270
5	SJRU-16	Ramgarh	Ramgarh, Samba	384
6	SJRU-19	Bhaderwah	Bhaderwah, Doda	1524
7	SJRU-20	Bhaderwah	Bhaderwah, Doda	1524
8	SJRU-24	Mamka	RS Pora, Jammu	270
9	SJRU-32	Doda	Doda	1107
10	SJRU-33	Gulahate	Rajouri	915
11	SJRU-38	Doongi	Pouni	466
12	SJRU-39	Sunderbani	Sunderbani, Rajouri	633
13	SJRU-41	Dadua	Reasi	466
14	SJRU-42	Keni Magyal	Poonch	1021
15	SJRU-47	Badakna	Thana Mandi, Poonch	1668
16	SJRU-49	Chhajjla Segi	Mankato, Poonch	1027
17	SJRU-53	Krishna Ghati Top	Mendhar, Poonch	981
18	SJRU-58	Nowshera	Nowshera, Rajouri	1575
19	SJRU-61	Nowshera Pull	Nowshera, Rajouri	1575
20	SJRU-65	Kossalian	Poonch	1006
21	SJRU-67	Gulpur	Poonch	1577
22	SJRU-69	Sanai	Poonch	1580
23	SJRU-71	Manjakote	Rajouri	1580
24	SJRU-72	Chowki Choura	Akhnoor, Jammu	301
25	SJRU-73	Jaganoo	Udhampur	662

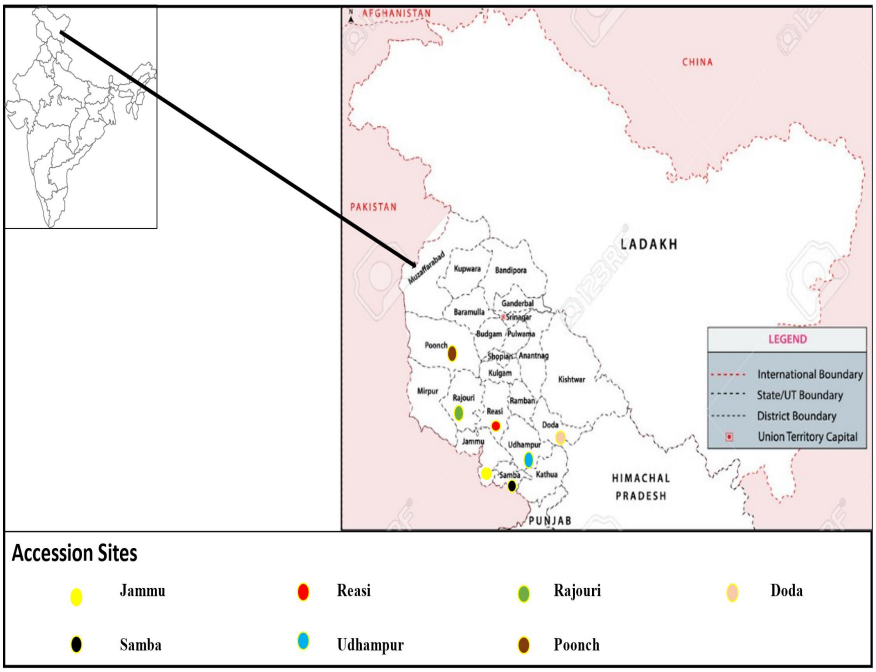


Figure 2: Map of Jammu and Kashmir depicting sources of germplasm collection

Plate 1(a). Layout of the experiment

Plate 1(b). Field view of the experiment

3.4.1 Morphological Parameters

3.4.1.1 Days to 50% germination

It was calculated by counting the number of days when 50% of the seeds in the plot showed germination from the date of sowing.

3.4.1.2 Plant height (cm)

The height of all five tagged plants was measured in centimeters from the base of the plant to the top of the plant at 100 days after sowing and the average plant height of each accession was worked out in centimeters.

3.4.1.3 Days to first leaf picking

The number of days taken from the date of sowing to the date of first picking were counted and their mean was calculated.

3.4.1.4 Number of pickings/plant

The total number of picking of leaves yielded from each accession at its vegetative growth stage and an average was worked out for each accession.

3.4.1.5 Number of leaves/plant

It is calculated by counting the total number of leaves picked per plant in each picking and their mean value was calculated.

3.4.1.6 Leaf length (cm)

The leaf length of five tagged plants was measured from the terminal end of the petiole up to the tip of the leaf blade using a graduated scale and their mean value was calculated in centimeters.

3.4.1.7 Leaf width (cm)

The leaf width of five tagged plants was measured with the help of a graduated scale. The leaf width was measured at the center of each leaf blade and was expressed in centimetre (cm) and their mean value was calculated.

3.4.1.8 Petiole length (cm)

The petiole length of five tagged plants were measured from the top of the petiole to the base of attachment with a stem with the help of a graduated scale and was expressed in centimetre (cm) and their mean value was calculated.

3.4.1.9 Green leaf yield/plot(kg) and per hectare(q)

The leaves picked from each plot were weighed in kilograms and readings were taken. The data from green leaf yield(kg) per plot is expressed in terms of green leaf yield per hectare (quintals per hectare).

$$\text{Green leaf yield per hectare (Q/ha)} = \frac{\text{Green leaf yield per plot (kg) x 10000 } (m^2)}{\text{Plot area x 100}}$$

3.4.1.10 Crop duration

The crop duration was recorded by calculating the number of days from the date of sowing to the date of seed harvest.

3.4.2 Bio-Chemical Parameters

3.4.2.1 Total phenolics

The total phenols were determined with the help of Folin-Ciocalteau reagent as method described by Singleton and Rossi, 1965.

Procedure:

1. Take 100 µl of extract and raise the volume to 1000 µL by adding 900 µL distilled water.
2. Add 1mL of 1 N Folin- Ciocalteu reagent and the reaction mixture was kept at room temperature for 5 min.
3. Add 3 mL of 20% Na_2CO_3 (sodium carbonate).
4. Keep them for incubation in dark at room temperature for 60 minutes.
5. Measure the absorbance of the reaction mixture at 760 nm using double beam UV-VIS spectrophotometer.
6. The total phenolic content was calculated from the equation obtained from the standard curve prepared from gallic acid (5 to 50 µg):

$$y = mx + c$$

The results were expressed as milligram gallic acid equivalents (mg GAE) per gram dry weight.

3.4.2.2 Total flavonoids

Flavonoid content was determined with the Aluminium chloride method by Christ and Muller, 1960.

Procedure:

1. Add 500µL of extract to 1mL of distilled water.
2. Add 75 µL of 5% sodium nitrite ($NaNO_2$) solution. Leave the reaction mixture undisturbed for approximately 6 min. at 25°C.
3. Then add 150 µL of 10% aluminium chloride ($AlCl_3$) to the solution. This was followed by 5 more minutes of undisturbed incubation at room temperature.
4. Treat the reaction mixture with 500 µL of 1 M NaOH.
5. Then the volume of reaction mixture was made up to 2.5 mL using distilled water.
6. The absorbance was measured at 510 nm using UV-VIS spectrophotometer.
7. Flavonoid content was calculated from the equation derived from the standard curve of catechin (5 to 50 µg):

$$y = mx + c$$

Results were expressed as mg of Quercetin equivalents (QE)/g dry weight.

3.4.2.3 Total carotenoids (Spectrophotometric by Lichtenthaler, 1987)

The total carotenoids was determined with the with help of method by Lichtenthaler, 1987.

Procedure:

1. Extract 5 g sample with acetone and a few crystals of Na_2SO_4.
2. Take extract in a screw cap culture tube and add 10%, 50 ml alcoholic KOH.
3. Heat on water bath for 30 min.
4. Take the material now in separating funnel and add 50 ml petroleum ether.
5. Remove the upper layer of petroleum ether containing pigments in 100 ml volumetric flask and make up the volume 100 ml with petroleum ether.
6. Record the O.D. at 452 nm.

Calculation

$$\text{Total carotene (mg/100 g)} = \frac{\text{O.D. x } 13.9 \text{ x } 10^4 \text{ x volume made up}}{\text{Weight of sample x } 560 \text{ x } 1000}$$

$$1 \text{ O.D.} = \frac{13.9 \text{ x } 10^4 \text{ g Beta-carotene}}{560}$$

3.4.2.4 DPPH free radical scavenging

The DPPH radical scavenging activity was determined according to the method of Spectrophotometric by Blois, 1958.

Procedure:

1. 1 mL of 0.5 mM methanol solution of DPPH was mixed with and to this 2 ml of 0.1 M sodium acetate buffer (pH 5.5) was added.
2. The reaction mixture was shaken and kept at room temperature in the dark for 30 min.
3. The absorbance was measured at 517 nm using UV-VIS spectrophotometer.
4. Methanol was used as a negative control. The radical scavenging activity (RSA) was calculated as a percentage of DPPH radical discoloration, using the equation:

$$\text{RSA (\%)} = (1 - A_{sample} / A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the positive control (containing all reagents except the test compound), and A_{sample} is the absorbance of the test sample.

3.4.2.5 Total Sugars (James B. Sumner, 1921)

The amount of total sugars was estimated using the anthrone method.

Anthrone method

Principle

Carbohydrates are dehydrated by conc. H_2SO_4, to form furfural. Furfural condenses with anthrone (10-keto- 9, 10 dihydroanthracene) to form a blue-green coloured complex which is measured colorimetrically at 630 nm.

Reagents

1. 2.5 N HCl.
2. Anthrone reagent: Dissolve 200 mg anthrone in 100ml of ice cold 95% H_2SO_4.

3. Standard glucose (stock): Dissolve 100mg in 100ml water.
4. Working standard: 10ml of stock diluted to 100ml with distilled water.

Method

1. Weigh 100mg of the sample into a boiling tube.
2. Hydrolyze by keeping it in a boiling water bath for 3 hours with 5ml of 2.5N HCL and cool to room temperature
3. Neutralize it with solid sodium carbonate until the effervescence ceases.
4. Make up the volume to 100ml and centrifuge.
5. Collect the supernatant and take 0.5ml and 1ml aliquot for analysis.
6. Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard. '0' serve as blank.
7. Make up the volume to 1ml in all the tubes including the samples tubes by adding distilled water.
8. Then add 4ml of anthrone reagent.
9. Heat for 8 min in a boiling water bath.
10. Cool rapidly and read the green to dark green colour at 630 nm.
11. Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis.
12. From the graph, calculate the amount of carbohydrates present in the sample tube.

Calculation:

$$\text{Amount of carbohydrate present in sample (\%)} = \frac{\text{Sugar value from graph (mg)}}{\text{Aliquot sample used (ml)}} \text{ X } \frac{\text{Total volume of extract(ml)}}{\text{Weight of sample (mg)}} \text{ X } 100$$

3. Visual Parameters

3.1. Leaf shape

Leaf shape was recorded on visual basis and each accession was categorized into following types as per following:

i. **Elliptical**: Leaves are widest in the middle, tapering on both ends.

ii. **Ovate**: Leaf is broadest below the middle and about two times as long as the width, also called oval (egg-shaped).

iii. **Lanceolate**: Leaf is three times or longer than width and broadest below the middle.

iv. **Oblong**: Leaf is two to three times as long as it is wide and has parallel sides.

v. **Cordate:** Heart-shaped.

vi. **Elliptical-cordate:** Elliptical towards the tip and cordate at junction with petiole.

3.2. Prominence of leaf veins

The presence or absence of leaf veins was observed in each accession through visual assessment at the time of leaf picking. Based on visual assessment, the leaves of accessions were classified into two categories, viz., Present (presence of prominent leaf veins) and Absent (absence of prominent leaf veins).

3.3. Spines in leaf axils

The leaf axils were examined for the presence or absence of spines through visual assessment and the accessions were classified into two categories, viz., Present (spines were present in leaf axils) and Absent (spines were absent in leaf axils).

3.4. Leaf pigmentation

The presence or absence of leaf pigmentation other than green color was observed in each accession at the time of leaf picking by their visual assessment. Based on this observation, accessions were classified into two categories, viz., Present (presence of pigmentation) and Absent (absence of pigmentation).

3.5. Leaf color

The leaf color of each accession was observed at the time of leaf picking through visual assessment. Based on the intensity of the green color of the leaves, they were classified into three categories as follows:

- ➢ Light Green
- ➢ Dull Green
- ➢ Dark Green

3.5 Statistical analysis

The averaged values of all the five plants of different genotypes were statistically analysed at the computer lab of Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha, Jammu, and were analysed in accordance with Gomez and Gomez (1984).

The analysis of variance was as follows:

Source of Variation	**d.f**	**Mean sum of squares (MSS)**	**Variance ratio (VR)**
Replications (r)	r-1	Mr	Mr/Me
Treatments (t)	t-1	Mt	Mt/Me
Error	(r-1)(t-1)	Me=Ve	
Total	**rt-1**	**Mr+Mt+Me**	

Where,

r = Number of replications

t = Number of treatments

Ve = Error of variance

For determining significance, the observed 'F' value was compared to the tabulated value. All of the characters that differed significantly were then used to estimate the following genetic parameters:

1. Coefficient of variability (phenotypic and genotypic)
2. Heritability
3. Genetic gain
4. Correlation coefficients
5. Path coefficients
6. Genetic diversity

3.5.1 Coefficients of variability

The genotypic and phenotypic coefficients of variability were calculated using the method proposed by Burton and De Vane (1953).

Genotypic coefficients of variability

$$GCV\ (\%) = (Vg/\bar{X}) \times 100$$

Phenotypic coefficients of variability

$$PCV\ (\%) = (Vp/\bar{X}) \times 100$$

where,

Vg = Genotypic variance (Mt-Me)/r

Vp = Phenotypic variance (Vg+Ve)

$\bar{X}$ = Population means.

PCV and GCV were classified as per Robinson *et al*. (1949)

0-10% - Low

10-20% - Moderate

20% and above – High

3.5.2 Heritability

Heritability in a broad sense was calculated as the ratio of genotypic variance to phenotypic variance and expressed in percentage (Allard, 1960 [a])

$$H\ (\%) = \frac{Vg}{Vp} \times 100$$

Where,

H = Heritability (%)

Vg = Genotypic variance (Mt-Me)/r

Vp = Phenotypic variance (Vg+Ve)

Heritability percentage was classified as per Robinson (1966)

0-30% - Low

30-60% - Moderate

60 and above - High

3.5.3 Genetic advance

The expected genetic advance (GA) resulting from the selection of five percent superior individuals was derived as per Allard (1960 [b]).

$$GA = H \times P \times K$$

where,

P = phenotypic standard deviation

K = 2.06 (Selection differential at 5 % selection intensity)

H = Heritability (%)

3.5.4 Genetic gain

Genetic advance expressed as per cent of the population mean was calculated using the formula proposed by Johnson *et al*. (1955 [a]).

$$GG = \frac{GA}{\bar{X}} \times 100$$

where,

GG = Genetic gain (%)

$\bar{X}$ = General mean of the character.

The genetic advance as per cent mean was classified as suggested by Johnson *et al.* (1955 a).

0-10% - Low

10-20% - Moderate

20% and above – High

3.5.5 Correlation

The genotypic and phenotypic correlation coefficients were calculated using the analysis of the variance-covariance table proposed by Al-Jibouri *et al*. (1958):

Source of Variation	**D.f**	**M.S.S**		**Mean sum of products**
		X	**Y**	
Replication	(r-1)	Mgx	Mgy	Mgxy
Accessions	(t-1)	Mex	Mey	Mexy
Error	(r-1)(t-1)			

where,

Genotypic covariance (CoVgxy) = (Mgxy-Mexy)/r

Phenotypic covariance (CoVpxy) = CoVgxy + CoVexy

3.5.5.1 Phenotypic coefficient of correlation

$$rp = \frac{CoVpxy}{\sqrt{(Vpx).(Vpy)}}$$

where,

CoVpxy = Phenotypic coefficient of variance between characters x & y

Vpx = Phenotypic variance of character x

Vpy = Phenotypic variance of character y

3.5.5.2 Genotypic coefficients of correlation

$$rg = \frac{CoVgxy}{\sqrt{(Vgx).(Vgy)}}$$

where,

CoVgxy = Genotypic coefficient of variance between characters x & y

Vgx = Genotypic variance of character x

Vgy = Genotypic variance of character y

Calculated correlation coefficients (r) were compared to tabulated ‘r’ values (Fisher and Yates, 1963) at (n-2) degree of freedom at 5% or 1% level of significance. The correlation was said to be significant if the calculated 'r' values at 5% were greater than the tabulated value of 'r'.

3.5.6 Path coefficient analysis

The genotypic and phenotypic correlation coefficients were calculated to determine their direct and indirect contribution to yield per plot.

Path coefficient analysis was performed to calculate the direct and indirect contribution of various characters to yield. Dewey and Lu (1959) method was used to obtain the direct and indirect effects. The path coefficients were determined by simultaneous selection the following equations, which express the fundamental relationship between genotypic correlation (r) and path coefficients (P).

$$r14 = P14 + P24r12 + P34r13$$

$$r24 = P14r21 + P24 + P34r23$$

$$r34 = P14r31 + P24r32 + P34$$

Where, r12, r24 and r34 are genotypic correlation of component characters with yield (dependent variable) and r12, r13, r23 are genotypic correlations among the component characters (independent variables).

The direct effect was estimated by the following set of equations:

$$P14 = C11r14 + C12r24 + C13r34$$

$$P24 = C21r14 + C22r24 + C23r34$$

$$P34 = C31r14 + C32r24 + C33r34$$

Where, C11, C12, C23 and C33 are constants calculated by using abbreviated Doulittle’s technique as described by Goulden (1959) and r12P34, r13P34, r23P34, r31P14 and r32P24 are indirect effects.

Residual effect:

The variation in the independent variable that remained undetermined after including all variables was assumed to be due to variable(s) on dependent variable was calculated as follows:

$$I = P2x4 + P214 + P224 + P234 + 2P14\ r12\ P24 + 2P14\ r13\ P34 + 2P24\ r23\ P34$$

3.5.7 Genetic diversity

3.5.7.1 Mahalanobis D^2 analysis

The genetic divergence among the test enteries was assessed using Mahalanobis' (1936) D^2 statistics analysis. The generalised distance between any two populations can be calculated using a formula.

$$D^2 = \Sigma\Sigma\ \lambda ij sisj$$

where,

D^2 = Square of generalized distance

λij = Reciprocal of common dispersal matrix

sai = (μi 1 - $\mu i2$)

saj = (μj 1- $\mu j2$)

μ = General mean

Since the computation formula requires higher order determinant inversion, the original correlated unstandardized character mean (Xs) was transformed to standardised uncorrelated variable (Ys) to simplify the computation procedure. The D^2 values were calculated by taking the corresponding uncorrelated (Ys) values of any two genotypes (Rao,1952).

3.5.7.2 Clustering of D^2 Values

Tocher's method was used to assign different accessions to different clusters. The two accessions with the shortest distance between them were considered first, followed by a third accession with the smallest average D^2 value from the first two varieties. The nearest fourth accession was then used, and the process was repeated until the average D^2 value was increased. The remaining accessions were then considered for the next cluster, and the process was repeated until all of the accessions were included in various clusters. The spatial distances

between clusters were calculated by taking the square root of the average intra and inter cluster D^2 values.

3.5.7.3 Intra cluster distance

The intra cluster distance were calculated by the formula given by Singh and Chaudhary (1977).

$$\text{Square of intra cluster distance} = \Sigma Di^2/n$$

where,

ΣDi^2 = the sum of distance between all possible combinations.

n = Number of all possible combinations.

3.5.7.4 Inter cluster distance

The inter-cluster distance were calculated using the formulae described by Singh and Chaudhary (1977).

$$\text{Square of inter cluster distance} = \Sigma Di^2/ninj$$

where,

ΣDi^2 is the sum of distances between every possible combinations (ninj) of the entries included in the cluster study.

ni = Number of entries in cluster i

nj = Number of entries in cluster j

3.5.7.5 Relative contribution of individual characters towards total divergences

The differences in uncorrelated means between all the characters were ranked for all pairwise combinations of accessions, with the highest differences receiving first rank. Finally, the percentage of first rank in a character was calculated to estimate its relative contribution to total divergence.

CHAPTER-IV

RESULTS

In the present investigation data with respect to morphological and bio-chemical characters were subjected to statistical and biometrical analysis and significance of results was verified. The mean findings are presented in this Chapter under following sub-headings:

4.1 Analysis of variance

4.2 Evaluation of accessions

4.3 Parameters of variability

4.4 Correlations

4.5 Path coefficient analysis

4.1 Analysis of variance

Analysis of variance for several characters showed significant differences for all the parameters under consideration is evident from the data (Appendix II).

4.2 Evaluation of accessions

4.2.1 Evaluation of rumex accessions for morpho-metrical characters

A perusal of data for morpho-metrical characters is presented in Table 4.1.

4.2.1.1 Days to 50% germination

A wide variation among accessions for days to 50% germination ranging between minimum 23.00 days (SJRU-53 & SJRU-65) to maximum 38.33 days (SJRU-04) with an overall general mean of 27.41. Among all accessions, the earliest 50% germination was recorded in the accession SJRU-53 (23.00 days) and SJRU-65 (23.00 days), which were statically at par with seven other accessions viz., SJRU-16 (23.33 days), SJRU-41 (23.33 days), SJRU-61 (23.33 days), SJRU-69 (23.33 days), SJRU-71 (23.33 days), SJRU-72 (23.33 days), and SJRU-33 (23.67 days). However, the highest days to 50% germination was recorded in the accession SJRU-04 (38.33 days).

4.2.1.2 Plant height (cm)

The mean performance of various accessions for plant height ranged from 22.00 cm (SJRU-20) to 42.20 cm (SJRU-72) with an overall general mean of 30.19 cm. The maximum plant height was recorded in accession SJRU-72 (42.20 cm), which was statistically at par with accession SJRU-33 (38.00 cm). However, the minimum plant height was recorded in accession SJRU-20 (22.00 cm).

4.2.1.3 Days to first leaf picking

Days to first leaf picking ranged from 70.66 days (SJRU-53 & SJRU-71) to 82.66 days (SJRU-04) with an overall general mean of 75.79 days. Among all accessions under study, the earliest days to first leaf picking were recorded in SJRU-53 (70.66 days) and SJRU-71 (70.66 days) which were statistically at par with accessions SJRU-72 (72.00 days), SJRU-61 (72.33 days), SJRU-16 (72.66 days) and SJRU-65 (74.00 days). However, the maximum days to first leaf picking was recorded in the accession SJRU-04 (82.66 days).

4.2.1.4 Number of pickings per plant

The data for number of pickings per plant varied from 2 days (SJRU-14, SJRU-16, SJRU-19, SJRU-32, SJRU-38, SJRU-41, SJRU-42, SJRU-49, SJRU-53 & SJRU-73) to 3 days (SJRU-04, SJRU-12, SJRU-20, SJRU-24, SJRU-33, SJRU-39, SJRU-47, SJRU-58, SJRU-61, SJRU-65, SJRU-67, SJRU-71 & SJRU-72) to 3 days with an overall general mean of 2.54. The maximum number of pickings per plant were recorded in accessions SJRU-04 (3.00), SJRU-12 (3.00), SJRU-20 (3.00), SJRU-24 (3.00), SJRU-33 (3.00), SJRU-39 (3.00), SJRU-47 (3.00), SJRU-58 (3.00), SJRU-61 (3.00), SJRU-65 (3.00), SJRU-67 (3.00), SJRU-71 (3.00) and SJRU-72 (3.00), which were found to be statistically at par with each other. However, the minimum number of pickings per plant were recorded in accessions viz., SJRU-14 (2.00), SJRU-16(2.00), SJRU-19 (2.00), SJRU-32 (2.00), SJRU-38 (2.00), SJRU-41 (2.00), SJRU-42 (2.00), SJRU-49 (2.00), SJRU-53 (2.00) & SJRU-73 (2.00) which were found to be statistically at par with each other.

4.2.1.5 Number of leaves per plant

The data for number of leaves per plant varied from 14.40 (SJRU-19) to 28.33 (SJRU-33) with an overall general mean of 20.57. The maximum number of leaves were recorded in accession SJRU-33 (28.33), which was found to be statistically at par with accession SJRU-61 (27.73) and SJRU-12 (27.00). However, the minimum number of leaves were recorded in accession SJRU-19 (14.40).

4.2.1.6 Leaf length (cm)

The mean performance of various accessions for leaf length ranged from 14.08 cm (SJRU-03) to 17.98 cm (SJRU-39) with an overall general mean of 16.50 cm. The maximum leaf length was recorded in accession SJRU-39 (17.98 cm), which was statistically at par with 9 accessions viz., SJRU-14 (17.66 cm), SJRU-73 (17.41 cm), SJRU-33 (17.40 cm), and SJRU-49 (17.32 cm) SJRU-04 (17.24 cm), SJRU-12 (17.12 cm), SJRU-53 (17.07 cm), SJRU-42 (17.03 cm) and SJRU-38 (16.92 cm). However, the minimum leaf length was recorded in accession SJRU-03 (14.08).

4.2.1.7 Leaf width (cm)

The mean performance of various accessions for leaf width varied from 4.89 cm (SJRU-69) to 6.99 cm (SJRU-39) with an overall general mean of 5.74 cm. The maximum leaf width was recorded in accession SJRU-39 (6.99 cm), which was statistically at par with accessions viz., SJRU-33 (6.63 cm) and SJRU-14 (6.49 cm). However, the minimum leaf width was recorded in accession SJRU-69 (4.89 cm).

4.2.1.8 Petiole length (cm)

The mean performance of various accessions for petiole length varied from 6.15 cm (SJRU-41 & SJRU-61) to 10.57 cm (SJRU-39) with an overall general mean of 8.07 cm. The maximum petiole length was recorded in accession SJRU-39 (10.57 cm), which was statistically at par with accessions viz., SJRU-42 (9.79 cm) and SJRU-38 (9.73 cm). However, the minimum (6.15 cm) petiole length was recorded in accession SJRU-41 & SJRU-61.

4.2.1.9 Green leaf yield/plot (kg) and per hectare (q/ha)

The mean performance of various accessions for green leaf yield/plot(kg) varies from 1.083 kg/plot (SJRU-19) to 3.996 kg/plot (SJRU-33) with an overall general mean of 2.21 kg/plot. Among all accessions, the maximum green leaf yield per plot was recorded in accession SJRU-33 (3.996 kg/plot), which was statistically at par with one accession namely, SJRU-12 (3.711 kg/plot). However, the minimum green leaf yield/plot(kg) was recorded in accession SJRU-19 (1.083 kg/plot).

The mean performance of various accessions for green leaf yield per hectare varies from 48.12 q/ha (SJRU-19) to 177.60 q/ha (SJRU-33) with an overall general mean of 98.37 q/ha. Among all accessions, the maximum green leaf yield per hectare was recorded in accession SJRU-33 (177.60 q/ha), which was statistically at par with accessions SJRU-12

Table 4.1 Evaluation of rumex accessions for morpho-metrical characters.

Accession(s)	Days to 50 percent germination	Plant height (cm)	Days to first leaf picking	Number of pickings/ plant	Number of leaves per plant	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Green leaf yield/plot (kg)	Yield per ha (q)	Crop duration
SJRU-03	34.33	22.80	80.33	2.33	19.86	14.08	5.10	6.81	1.651	73.36	171.00
SJRU-04	38.33	28.90	82.66	3.00	20.46	17.24	6.27	8.83	2.531	112.47	171.33
SJRU-12	26.67	28.90	76.33	3.00	27.00	17.12	6.33	9.14	3.711	164.92	166.00
SJRU-14	34.33	29.20	78.66	2.00	18.60	17.66	6.49	9.48	1.776	78.93	170.67
SJRU-16	23.33	27.40	72.66	2.00	17.73	16.21	5.65	8.27	1.472	65.42	161.00
SJRU-19	31.33	24.90	78.33	2.00	14.40	15.68	5.61	7.65	1.083	48.12	171.00
SJRU-20	34.00	22.00	79.66	3.00	23.06	16.57	5.61	6.86	2.996	133.16	171.67
SJRU-24	26.67	27.60	75.33	3.00	23.73	16.01	5.39	6.72	3.533	157.04	166.33
SJRU-32	31.33	28.40	78.00	2.00	17.20	15.94	5.23	8.57	1.375	61.10	166.00
SJRU-33	23.67	38.00	74.33	3.00	28.33	17.40	6.63	8.37	3.996	177.60	161.00
SJRU-38	26.00	24.80	75.66	2.00	19.40	16.92	6.33	9.73	2.137	94.99	166.67
SJRU-39	26.00	31.60	75.00	3.00	21.00	17.98	6.99	10.57	2.201	97.84	166.33
SJRU-41	23.33	31.30	74.33	2.00	17.06	14.85	5.06	6.15	1.444	64.18	166.00
SJRU-42	26.00	29.60	75.33	2.00	18.33	17.03	5.92	9.79	1.840	81.78	166.00
SJRU-47	26.67	29.20	77.33	3.00	21.46	16.74	5.67	8.92	1.793	79.70	166.00
SJRU-49	26.33	33.93	75.66	2.00	17.66	17.32	5.77	6.50	1.429	63.53	166.00
SJRU-53	23.00	30.66	70.66	2.00	15.60	17.07	5.61	7.46	1.584	70.40	161.00
SJRU-58	26.33	30.42	75.00	3.00	21.33	16.80	5.86	8.13	2.164	96.18	166.67
SJRU-61	23.33	25.60	72.33	3.00	27.73	15.67	5.46	6.15	3.604	160.18	161.00
SJRU-65	23.00	36.97	74.00	3.00	20.20	16.05	5.59	8.78	1.992	88.53	161.67
SJRU-67	26.67	33.80	74.66	3.00	22.40	16.08	5.23	6.60	2.464	109.51	161.67
SJRU-69	23.33	35.42	74.33	2.33	19.50	15.93	4.89	7.48	2.545	113.13	161.33
SJRU-71	23.33	30.60	70.66	3.00	23.20	16.15	5.50	7.21	1.639	72.83	161.00
SJRU-72	23.33	42.20	72.00	3.00	23.73	16.63	5.87	8.44	3.232	143.64	161.00
SJRU-73	34.67	30.53	80.00	2.00	15.40	17.41	5.67	9.18	1.141	50.73	171.33
Mean	**27.41**	**30.19**	**75.79**	**2.54**	**20.57**	**16.50**	**5.74**	**8.07**	**2.21**	**98.37**	**165.59**
C.D. (5%)	**1.814**	**4.577**	**3.483**	**0.272**	**1.760**	**1.232**	**0.523**	**0.902**	**0.257**	**12.962**	**2.435**
SE(m) ±	**0.636**	**1.553**	**1.221**	**0.095**	**0.767**	**0.432**	**0.183**	**0.316**	**0.055**	**4.441**	**0.954**
SE(d)	**0.899**	**1.782**	**1.727**	**0.135**	**0.977**	**0.611**	**0.259**	**0.447**	**0.078**	**5.452**	**1.308**
C.V.	**5.018**	**5.173**	**5.791**	**6.47**	**2.245**	**4.535**	**5.528**	**6.789**	**6.298**	**6.298**	**4.893**

Plate 2. Recording of morphological characters

Table 4.2 Evaluation of rumex accessions for bio-chemical characters.

Accession(s)	Total Phenolics (mg GAE/g)	Total flavonoids (mg QE/g)	Total carotenoids (mg/100g)	DPPH free radical scavenging (%)	Total sugar (%)
SJRU-03	35.95	08.57	7.97	85.64	08.24
SJRU-04	34.01	07.64	8.40	82.84	07.94
SJRU-12	45.43	10.24	8.73	95.98	10.08
SJRU-14	34.78	07.94	8.20	84.23	08.12
SJRU-16	40.96	09.34	6.97	90.12	09.18
SJRU-19	30.12	06.86	6.53	79.44	07.24
SJRU-20	37.57	08.76	7.37	86.56	08.45
SJRU-24	42.32	09.72	7.27	92.97	09.56
SJRU-32	29.89	07.01	6.33	79.59	07.54
SJRU-33	46.96	10.08	8.83	96.44	10.26
SJRU-38	38.95	09.01	6.83	88.78	08.99
SJRU-39	39.34	09.17	6.73	87.83	08.75
SJRU-41	33.76	07.32	6.63	82.12	07.08
SJRU-42	41.32	09.46	8.27	91.22	09.42
SJRU-47	38.03	08.82	7.33	87.06	08.87
SJRU-49	41.44	09.58	6.90	89.76	09.04
SJRU-53	41.57	09.62	7.70	90.34	09.30
SJRU-58	34.26	07.83	7.90	83.65	08.06
SJRU-61	44.47	09.93	8.97	95.21	10.46
SJRU-65	35.01	08.36	8.23	85.44	08.37
SJRU-67	32.44	07.03	7.57	80.67	07.38
SJRU-69	34.21	07.74	8.60	83.28	07.82
SJRU-71	38.67	08.96	7.13	88.34	08.68
SJRU-72	43.84	09.86	8.53	94.79	09.83
SJRU-73	30.47	06.92	6.23	78.07	07.64
Mean	**38.51**	**08.63**	**7.60**	**87.21**	**08.65**
C.D. (5%)	**1.501**	**0.363**	**0.29**	**3.989**	**0.376**
SE(m) ±	**0.526**	**0.127**	**0.10**	**1.398**	**0.132**
SE(d)	**0.744**	**0.180**	**0.14**	**1.978**	**0.186**
C.V.	**2.410**	**2.553**	**2.313**	**2.777**	**2.640**

Plate 3. Recording of bio-chemical characters

(164.92 q/ha). However, the minimum green leaf yield per hectare was recorded in accession SJRU-19 (48.12 q/ha).

4.2.1.10 Crop duration

The mean performance of various accessions for crop duration ranged from 161.00 days (SJRU-16, SJRU-33, SJRU-53, SJRU-61, SJRU-71 & SJRU-72) to 171.67 days (SJRU-20) with an overall general mean of 165.59 days. Among all accessions, the earliest days for crop duration (161.00 days) was recorded in six accessions viz., SJRU-16, SJRU-33, SJRU-53, SJRU-61, SJRU-71 and SJRU-72, which were statistically at par with 3 accessions viz., SJRU-69 (161.33 days), SJRU-65 (161.67 days) and SJRU-67 (161.67 days). However, the longest crop duration was recorded in SJRU-20 (171.67 days).

4.2.2 Evaluation of rumex accessions for bio-chemical characters

A perusal of data for bio-chemical characters is presented in Table 4.2.

4.2.2.1 Total phenolics (mg GAE/g)

The mean performance of various accessions for total phenolics varied from 29.89 mg GAE/g (SJRU-32) to 46.96 mg GAE/g (SJRU-33) with an overall general mean of 38.51 mg GAE/g. Among all the accessions, the highest total phenol content was recorded in accession SJRU-33 (46.96 mg GAE/g), which was statistically at par with accession SJRU-12 (45.43 mg GAE/g). However, the minimum total phenol content was recorded in accession SJRU-32 (29.89 mg GAE/g).

4.2.2.2 Total flavonoids (mg QE/g)

The mean performance of various accessions for total flavonoids varies from 6.86 mg QE/g (SJRU-19) to 10.24 mg QE/g (SJRU-12) with an overall general mean of 8.63 mg QE/g. The maximum total flavonoids were observed in accession SJRU-12 (10.24 mg QE/g), which was statistically at par with two accessions viz., namely SJRU-33 (10.08 mg QE/g) and SJRU-61 (9.93 mg QE/g). However, the lowest total flavonoids were observed in accession SJRU-19 (6.86 mg QE/g).

4.2.2.3 Total carotenoids (mg/100g)

The data recorded for total carotenoids ranged from 6.23 mg/100g (SJRU-73) to 8.97 mg/100g (SJRU-61) with an overall general mean of 7.60 mg/100g. The maximum total carotenoid content was observed in accession SJRU-61 (8.97 mg/100g), which was statistically

at par with 2 accessions SJRU-33 (8.83 mg/100g) and SJRU-12 (8.73 mg/100g). However, the minimum total carotenoids were recorded in SJRU-73 (6.23 mg/100g).

4.2.2.4 DPPH free radical scavenging (%)

The data for DPPH free radical scavenging varied from 78.07% (SJRU-73) to 96.44% (SJRU-33) with an overall general mean of 87.21%. Among all accessions, the maximum DPPH free radical scavenging was observed in accession SJRU-33 (96.44%), which was statistically at par with 4 accessions viz., SJRU-12 (95.98%), SJRU-61 (95.21%), SJRU-72 (94.79%) and SJRU-24 (92.97%). However, the minimum DPPH free radical scavenging was recorded in the accession SJRU-73 (78.07%).

4.2.2.5 Total sugar (%)

The data for total sugar content varied from 7.08% (SJRU-41) to 10.46% (SJRU-61) with an overall general mean of 8.65%. The maximum total sugar was observed in accession SJRU-61(10.46%), which was statistically at par with two accessions viz., SJRU-33 (10.26%) and SJRU-12 (10.08%). However, the minimum was recorded in the accession SJRU-41 (7.08%).

4.2.3 Evaluation of rumex accessions for visual characters

A perusal of data for visual characters is presented in Table 4.3.

4.2.3.1 Leaf shape type

The leaves of rumex were classified into 6 categories for leaf shape viz., cordate, elliptical, ovate, lanceolate, elliptical-cordate and oblanceolate. Eight accessions were elliptical in shape (SJRU-03, SJRU-04, SJRU-16, SJRU-38, SJRU-53, SJRU-67, SJRU-69 and SJRU-71), seven accessions were lanceolate in shape (SJRU-12, SJRU-49, SJRU-58, SJRU-61, SJRU-65, SJRU-72, SJRU-73), five accessions were oblanceolate in shape (SJRU-14, SJRU-24, SJRU-41, SJRU-42, SJRU-47), three accessions were ovate in shape (SJRU-19, SJRU-20 and SJRU-32), one accession was cordate in shape (SJRU-33) and one accession was elliptical-cordate in shape (SJRU-39).

4.2.3.2 Prominence of leaf veins

Based on visual assessment of the leaves, all twenty-five accessions of rumex showed the presence of prominent leaf veins.

Table 4.3 Evaluation of rumex accessions for visual characters.

Accession(s)	Leaf shape type	Prominence of leaf veins	Spines in leaf axils	Leaf pigmentation	Leaf color
SJRU-03	Elliptical	Present	Absent	Absent	Dull Green
SJRU-04	Elliptical	Present	Absent	Absent	Dull Green
SJRU-12	Lanceolate	Present	Absent	Absent	Dark Green
SJRU-14	Oblanceolate	Present	Absent	Absent	Dull Green
SJRU-16	Elliptical	Present	Absent	Absent	Dull Green
SJRU-19	Ovate	Present	Absent	Absent	Dull Green
SJRU-20	Ovate	Present	Absent	Absent	Dark Green
SJRU-24	Oblanceolate	Present	Absent	Absent	Dull Green
SJRU-32	Ovate	Present	Absent	Absent	Light Green
SJRU-33	Cordate	Present	Absent	Absent	Dark Green
SJRU-38	Elliptical	Present	Absent	Absent	Light Green
SJRU-39	Elliptical-cordate	Present	Absent	Absent	Dull Green
SJRU-41	Oblanceolate	Present	Absent	Absent	Light Green
SJRU-42	Oblanceolate	Present	Absent	Absent	Light Green
SJRU-47	Oblanceolate	Present	Absent	Absent	Dull Green
SJRU-49	Lanceolate	Present	Absent	Absent	Dull Green
SJRU-53	Elliptical	Present	Absent	Absent	Light Green
SJRU-58	Lanceolate	Present	Absent	Absent	Dark Green
SJRU-61	Lanceolate	Present	Absent	Absent	Light Green
SJRU-65	Lanceolate	Present	Absent	Absent	Dull Green
SJRU-67	Elliptical	Present	Absent	Absent	Light Green
SJRU-69	Elliptical	Present	Absent	Absent	Dull Green
SJRU-71	Elliptical	Present	Absent	Absent	Dull Green
SJRU-72	Lanceolate	Present	Absent	Absent	Dark Green
SJRU-73	Lanceolate	Present	Absent	Absent	Dark Green

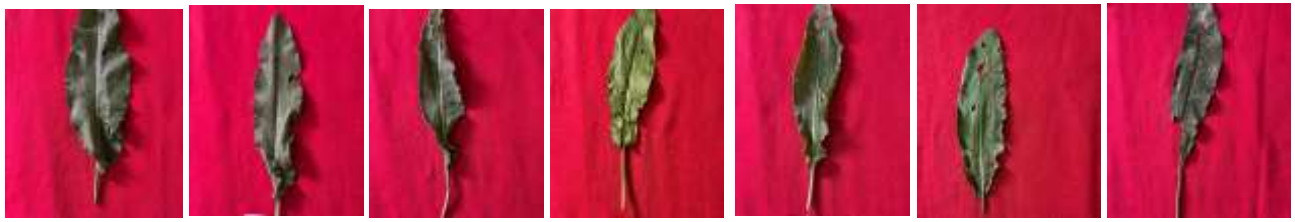
(a) Elliptical shaped

SJRU-03 SJRU-04 SJRU-16 SJRU-38 SJRU-53 SJRU-67 SJRU-69 SJRU-71

(b) Cordate shaped

SJRU-33

(c) Lanceolate shaped



SJRU-12 SJRU-49 SJRU-58 SJRU-61 SJRU-65 SJRU-72 SJRU-73

(d) Elliptical-cordate

SJRU-39

(e) Oblanceolate shaped

SJRU-14 SJRU-24 SJRU-41 SJRU-42 SJRU-47

(f) Ovate shaped

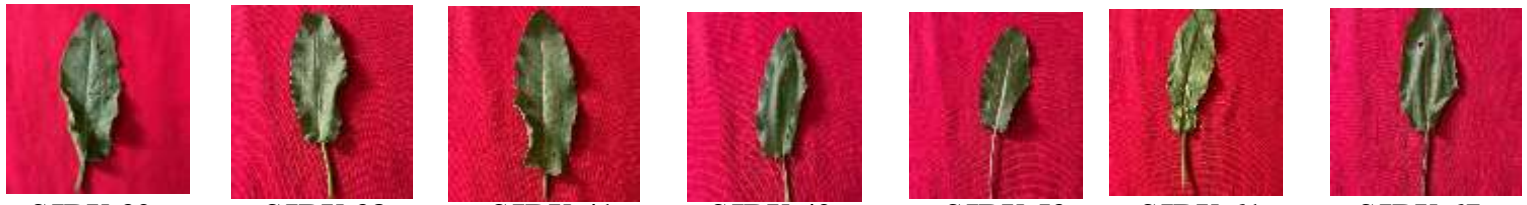
SJRU-19 SJRU-20 SJRU-32

Plate 4. Classification of accessions based on leaf shape type

(a) Dull green-colored leaves

SJRU-03 SJRU-04 SJRU-14 SJRU-16 SJRU-19 SJRU-24 SJRU-39 SJRU-47 SJRU-49 SJRU-65 SJRU-69 SJR71

(b) Light green colored leaves



SJRU-32 SJRU-38 SJRU-41 SJRU-42 SJRU-53 SJRU-61 SJRU-67

(c) Dark green colored leaves

SJRU-12 SJRU-20 SJRU-33 SJRU-58 SJRU-72 SJRU-73

Plate 5. Classification of accessions based on leaf color

Table 4.4 Genetic variation in *Rumex spp.* evaluated under sub-tropical conditions of Jammu.

Character(s)	Mean ± SE(m)	Range	Coefficient of variability		Heritability (bs) (%)	Genetic advance	Genetic advance as %age of population mean
			PCV	GCV			
Days to 50% germination	27.41 ± 0.64	23.00-38.33	17.05	16.57	94.45	9.10	33.18
Plant height (cm)	30.19 ± 1.55	22.00-42.20	15.82	15.49	95.98	9.44	31.27
Days to first leaf picking	75.79 ± 1.22	70.66-82.66	4.68	03.76	64.50	4.72	06.22
Number of pickings/plant	02.54 ± 0.095	2.00-3.00	19.94	18.86	89.45	0.94	36.74
Number of leaves per plant	20.57 ± 0.77	14.40-28.33	18.18	18.05	98.48	7.59	36.89
Leaf length (cm)	16.50 ± 0.43	14.08-17.98	06.56	04.74	52.17	1.16	7.05
Leaf width (cm)	05.74 ± 0.18	04.89-6.99	10.07	08.41	69.85	0.83	14.49
Petiole length (cm)	08.07 ± 0.32	06.15-10.57	16.39	14.91	82.84	2.26	27.96
Green leaf yield/plot (kg)	02.21 ± 0.06	01.083-3.99	38.76	38.52	98.78	1.75	78.86
Yield per hectare(q)	98.37 ± 4.44	48.12-177.60	38.76	38.52	98.77	77.57	78.85
Crop duration	165.59 ± 0.95	161.00-171.67	02.47	02.30	86.94	7.33	4.43
Total phenolics (mg GAE/g)	38.51 ± 0.53	29.89-46.96	13.13	12.90	96.63	9.88	26.13
Total flavonoids (mg QE/g)	08.63 ± 0.13	06.86-10.24	12.84	12.58	96.04	2.19	25.40
Total carotenoids (mg/100g)	07.60 ± 0.10	06.23-8.97	11.16	10.92	95.83	1.70	22.02
DPPH free radical scavenging (%)	87.21 ± 1.40	78.07-96.44	06.57	05.96	82.13	9.70	11.12
Total sugar (%)	08.65 ± 0.13	07.08-10.46	11.32	11.00	94.56	1.91	22.04

4.2.3.3 Spines in leaf axils

Based on visual assessment of the leaf axils, all the twenty-five accessions of rumex showed the absence of leaf axils.

4.2.3.4 Leaf pigmentation

Based on visual assessment of the leaves, all the twenty-five accessions of rumex showed the absence of leaf pigmentation.

4.2.3.5 Leaf color

The leaves of rumex were classified into 3 categories for leaf color viz., light-green, dull-green and dark-green through visual assessment. Twelve accessions were dull-green in color (SJRU-03, SJRU-04, SJRU-14, SJRU-16, SJRU-19, SJRU-24, SJRU-39, SJRU-47, SJRU-49, SJRU-65, SJRU-69, SJRU-71), seven accessions were light-green in color (SJRU-32, SJRU-38, SJRU-41, SJRU-42, SJRU-53, SJRU-61, SJRU-67) and six accessions were dark-green in color (SJRU-12, SJRU-20, SJRU-33, SJRU-58, SJRU-72, SJRU-73).

4.3 Parameters of variability

The estimates of coefficient of variability (phenotypic and genotypic), heritability (broad sense), genetic advance and genetic advance as percentage of mean were worked out to facilitate selection for various characters (Table 4.4).

4.3.1 Coefficients of variability

The phenotypic coefficient of variability (PCV) was in general higher in magnitude than the corresponding genotypic coefficient of variability (GCV) for all characters under study. The phenotypic and genotypic coefficient of variability were found to be high for green leaf yield/plot (38.76% and 38.52%) and yield per hectare (38.76% and 38.52%). The phenotypic coefficient of variability (PCV) and genotypic coefficient of variability (GCV) were found to be moderate for number of pickings/plant (19.94% and 18.86%), number of leaves per plant (18.18% and 18.05%), days to 50% germination (17.05% and 16.57%), petiole length (16.39% and 14.91%), plant height (15.82% and 15.49%), total phenolics (13.13% and 12.90%), total flavonoids (12.84% and 12.58%), total sugar (11.32% and 11.00%) and total carotenoids (11.16% and 10.92%). Low phenotypic coefficient and genotypic coefficient of variability were observed for crop duration (2.47% and 2.30%), days to first leaf picking (4.68% and 3.76%), leaf length (6.56% and 4.74%), DPPH free radical scavenging (6.57% and 5.96%) and leaf width (10.07% and 8.41%).

4.3.2 Heritability

Heritability (broad sense) ranged from moderate (52.17%) for leaf length to high (98.78%) for green leaf yield/plot, which is followed by yield per hectare (98.77%), number of leaves per plant (98.48%), total phenolics (96.63%), total flavonoids (96.04%), plant height (95.98%), total carotenoids (95.83%), total sugar (94.56%), days to 50% germination (94.45%), number of pickings/plant (89.45%), crop duration (86.94%), petiole length (82.84%), DPPH free radical scavenging (82.13%), leaf width (69.85%) and days to first leaf picking (64.50%).

4.3.3 Genetic gain

The genetic gain (genetic advance expressed as per cent of population mean) ranged from low (4.43%) to high (78.86%). High genetic gain as percentage of mean were observed for green leaf yield/plot (78.86%), yield per hectare (78.85%), number of leaves per plant (36.89%), number of pickings/plant (36.74%), days to 50% germination (33.18%), plant height (31.27%), petiole length (27.96), total phenolics (26.13%), total flavonoids (25.40%), total sugar (22.04%) and total carotenoids (22.02%). Moderate genetic gain was observed for leaf width (14.49%) and DPPH free radical scavenging (11.12%). Lowest genetic gain was observed for crop duration (4.43%), days to first leaf picking (6.22%) and leaf length (7.05%).

4.4 Correlation studies

Phenotypic and genotypic correlation among different characters in *Rumex spp.* were computed and are presented in Tables 4.5 and 4.6. The result showed that genotypic correlation coefficients were high in magnitude than the phenotypic correlation for almost all characters under study.

4.4.1 Phenotypic correlation

Phenotypic correlation among different characters showed that days to 50% germination was negatively and significantly correlated with plant height (-0.479), number of leaves per plant (-0.262), total phenolics (-0.244) and total sugars (-0.234) whereas, it was positively and significantly correlated with days to first leaf picking (0.668) and crop duration (0.818). Plant height was negatively and significantly correlated with crop duration (-0.564), whereas, it was positively and significantly correlated with leaf length (0.237) and total phenolics (0.263). Days to first leaf picking was positively and significantly correlated with crop duration (0.743). Number of pickings per plant was positively and significantly correlated with number of leaves per plant (0.790) and green leaf yield per hectare (0.506). Number of

Table 4.5 Phenotypic correlation coefficients among different morpho-metrical and bio-chemical characters in *Rumex spp.*

Character(s)	PH	DFLP	NPP	NLP	LL	LW	PL	CD	TP	TF	TC	DFRS	TS	GLYPH
DFG	-0.479 *	0.668 **	-0.130	-0.262 *	0.066	0.086	0.144	0.818 **	-0.244 *	-0.192	0.194	-0.223	-0.234*	-0.054
PH		-0.198	0.202	0.163	0.237 *	0.107	0.106	-0.564 **	0.263 *	0.215	0.216	0.220	0.197	-0.068
DFLP			0.043	-0.084	0.036	0.118	0.161	0.743 **	0.003	0.032	0.196	-0.005	-0.026	-0.158
NPP				0.790 **	0.043	0.163	-0.048	-0.199	0.165	0.177	0.302	0.163	0.108	0.506 **
NLP					0.048	0.224	-0.104	-0.324 **	0.247	0.214	0.299	0.227	0.213	0.737 **
LL						0.764 **	0.599 **	0.046	0.159	0.154	-0.120	0.117	0.094	0.370
LW							0.699 **	0.126	0.280 *	0.265 *	-0.008	0.247 *	0.239 *	0.250
PL								0.171	0.015	0.087	-0.019	-0.014	-0.006	-0.059
CD									-0.314 **	-0.264*	-0.304*	-0.318 **	-0.309 **	-0.158
TP										0.956**	0.313**	0.925 **	0.936 **	0.259
TF											0.282 *	0.913 **	0.933 **	0.198
TC												0.359**	0.368 **	0.216
DFRS													0.892 **	0.204
TS														0.308

***Significant at 5% level of significance **Significant at 1% level of significance**

Note**: DFG**=Days to 50% germination, **PH**=Plant height (cm), **DFLP**=Days to first leaf picking, **NPP**= Number of pickings per plant, **NLP**= Number of leaves per plant, **LL**=Leaf length (cm), **LW**=Leaf width (cm), **PL**=Petiole length (cm), **CD**=Crop duration, **TP**=Total phenolics (mg GAE/g), **TF**=Total flavonoids (mg QE/g), **TC**=Total carotenoids (mg/100g), **DFRS**=DPPH free radical scavenging (%), **TS**=Total sugar (%), **GLYPH**=Green leaf yield per hectare (q)

Table 4.6 Genotypic correlation coefficients among different morpho-metrical and bio-chemical characters in *Rumex spp.*

Character(s)	PH	DFLP	NPP	NLP	LL	LW	PL	CD	TP	TF	TC	DFRS	TS	GLYPH
DFG	-0.501*	0.874**	-0.142	-0.268	0.077	0.119	0.165	0.926**	-0.264	-0.213	0.210	-0.252	-0.259	-0.052
PH		-0.224	0.242	0.171	0.306	0.117	0.120	-0.599**	0.275	0.235	0.299	0.265	0.214	-0.065
DFLP			0.024	-0.118	0.155	0.246	0.225	0.838**	0.020	0.052	0.233	0.039	-0.019	-0.189
NPP				0.826 **	0.123	0.209	-0.062	-0.248	0.112	0.124	0.315	0.166	0.158	0.517 **
NLP					0.088	0.267	-0.114	-0.365	0.266	0.230	0.291	0.241	0.216	0.745 **
LL						0.837 **	0.702 **	0.070	0.192	0.235	0.069	0.227	0.142	0.373
LW							0.735 **	0.178	0.331	0.352	0.118	0.360	0.304	0.254
PL								0.193	0.001	0.093	0.001	0.043	0.005	-0.083
CD									-0.335	-0.292	-0.315	-0.307	-0.351	-0.172
TP										0.914 **	0.397*	0.984 **	0.972**	0.282
TF											0.339	0.963**	0.966 **	0.215
TC												0.445 *	0.450*	0.246
DFRS													0.921**	0.227
TS														0.234

***Significant at 5% level of significance **Significant at 1% level of significance**

Note**: DFG**=Days to 50% germination, **PH**=Plant height (cm), **DFLP**=Days to first leaf picking, **NPP**= Number of pickings per plant, **NLP**= Number of leaves per plant, **LL**=Leaf length (cm), **LW**=Leaf width (cm), **PL**=Petiole length (cm), **CD**=Crop duration, **TP**=Total phenolics (mg GAE/g), **TF**=Total flavonoids (mg QE/g), **TC**=Total carotenoids (mg/100g), **DFRS**=DPPH free radical scavenging (%), **TS**=Total sugar (%), **GLYPH**=Green leaf yield per hectare (q)

leaves per plant was negatively and significantly correlated with crop duration (-0.324), whereas it was positively and significantly correlated with green leaf yield per hectare (0.737). Leaf length was positively and significantly correlated with leaf width (0.764) and petiole length (0.599). Leaf width was positively and significantly correlated with petiole length (0.699), total phenolics (0.280), total flavonoids (0.265), DPPH free radical scavenging (0.247) and total sugars (0.239). Crop duration was negatively and significantly correlated with total phenolics (-0.314), total flavonoids (-0.264), total carotenoids (-0.304), DPPH free radical scavenging (-0.318) and total sugars (-0.309). Total phenolics were positively and significantly correlated with total flavonoids (0.956), DPPH free radical scavenging (0.925), total sugars (0.936) and total carotenoids (0.313). Total flavonoids were positively and significantly correlated with DPPH free radical scavenging (0.913), total sugars (0.933) and total carotenoids (0.282). Total carotenoids were positively and significantly correlated with DPPH free radical scavenging (0.359) and total sugars (0.368). DPPH free radical scavenging was positively and significantly correlated with total sugars (0.892).

4.4.2 Genotypic correlation

Genotypic correlation followed the same trend as was observed at phenotypic levels among different characters. Days to 50% germination was negatively and significantly correlated with plant height (-0.501), whereas, it was positively and significantly correlated with days to first leaf picking (0.874) and crop duration (0.926). Plant height was negatively and significantly correlated with crop duration (-0.599). Days to first leaf picking was positively and significantly correlated with crop duration (0.838). Number of pickings per plant was positively and significantly correlated with number of leaves per plant (0.826) and green leaf yield per hectare (0.517). Number of leaves per plant was positively and significantly correlated with green leaf yield per hectare (0.745). Leaf length was positively and significantly correlated with leaf width (0.837) and petiole length (0.702). Leaf width was positively and significantly correlated with petiole length (0.735). Total phenolics was positively and significantly correlated with total flavonoids (0.914), DPPH free radical scavenging (0.984), total sugars (0.972) and total carotenoids (0.397). Total flavonoids was positively and significantly correlated with DPPH free radical scavenging (0.963) and total sugars (0.966). Total carotenoids was positively and significantly correlated with DPPH free radical scavenging (0.445) and total sugars (0.450). DPPH free radical scavenging was positively and significantly correlated with total sugars (0.921).

4.5 Path coefficient analysis

Path coefficient was estimated for all yield attributing characteristics to quantify the direct and indirect link of one variable (cause) through another on the end product (effect). Green leaf yield correlation coefficients with relevant variables were divided into direct and indirect impacts. Important characteristics, such as green leaf yield per plot and per hectare were included as dependent variables in this study. The direct and indirect effects of component traits on *Rumex spp.* production are described further below.

Genotypic path analysis (Table 4.5) revealed that maximum direct effect on green leaf yield was exerted by number of leaves per plant (0.9373) followed by number of pickings per plant (0.2066), leaf length (0.0530), total sugar (0.0503), leaf width (0.0439), DPPH free radical scavenging (0.0437) and plant height (0.0103) while maximum negative direct effect was shown by total flavonoids (-0.2911) followed by total phenolics (-0.2490), petiole length (-0.1381), days to 50% germination (-0.1372), crop duration (-0.0402), days to first leaf picking (-0.0302) and total carotenoids (-0.0110). Maximum positive indirect towards green leaf yield per plot and per hectare was shown by leaf length via number of leaves per plant (0.4042), followed by leaf width via number of leaves per plant (0.2877), whereas maximum negative indirect effect was shown DPPH free radical scavenging via total flavonoids (-0.2845) followed by total flavonoids via total phenol (-0.2843).

4.6 Genetic divergence

The quantitative estimation of genetic divergence was made by adopting Mahalanobis D^2 statistics for morpho-metrical and bio-chemical characters.

4.6.1 D^2 statistics for morpho-metrical characters

The data analysed for genetic diversity using D^2 statistics for morpho-metrical characters is presented in Tables 4.8, 4.9, 4.10, 4.11 and 4.12.

4.6.1.1 Clustering of accessions into different clusters

Based on D^2statistics values, 25 accessions of rumex were grouped into seven clusters using Tochers method (Rao,1952) as shown in the dendrogram in Figure 3. Cluster 1 had the highest number of accessions i.e. 12, namely SJRU-41, SJRU-53, SJRU-16, SJRU-49, SJRU-42, SJRU-47, SJRU-58, SJRU-39, SJRU-65, SJRU-14, SJRU-38 and SJRU-03. Cluster 2 only 1 accession namely SJRU-71. Cluster 3 had 2 number of accessions namely SJRU-19 and

Table 4.7 Path coefficient analysis showing direct and indirect effects of various characters on green leaf yield in *Rumex spp.*

Character(s)	DFG	PH	DFLP	NPP	NLP	LL	LW	PL	CD	TP	TF	TC	DFRS	TS	GLYPH
DFG	**-0.1372**	0.0677	0.0189	-0.1088	0.0364	-0.0098	-0.0145	-0.0215	-0.1218	0.0352	0.0283	-0.0281	0.0331	0.0343	-0.052
PH	-0.0051	**0.0103**	0.0023	-0.0022	0.0017	0.0028	0.0012	0.0012	-0.0060	0.0028	0.0023	-0.0024	0.0026	0.0021	-0.065
DFLP	0.0042	-0.0069	**-0.0302**	-0.0016	-0.0246	-0.0027	-0.0058	0.0017	0.0070	-0.0210	-0.0214	0.0222	-0.0220	-0.0224	-0.189
NPP	0.1638	-0.0444	0.0107	**0.2066**	-0.0179	0.0216	0.0438	0.0413	0.1644	0.0072	0.0137	-0.0134	0.0087	0.0004	0.517
NLP	-0.3093	0.3187	0.9337	0.0542	**0.9373**	0.4042	0.2877	0.0275	-0.3995	0.0097	0.0536	-0.0709	0.0828	0.0067	0.745
LL	0.0204	-0.0233	-0.0731	-0.0065	-0.0852	**0.0530**	-0.0319	-0.0047	0.0275	-0.0986	-0.0983	0.0996	-0.0987	-0.0978	0.373
LW	0.0046	0.0049	0.0082	0.0091	0.0108	0.0345	**0.0439**	0.0311	0.0068	0.0134	0.0137	-0.0138	0.0136	0.0120	0.254
PL	-0.0216	-0.0159	0.0078	-0.0276	0.0151	-0.0902	-0.0995	**-0.1381**	-0.0254	-0.0008	-0.0125	0.0065	-0.0030	-0.0002	-0.083
CD	-0.0357	0.0236	0.0093	-0.0320	0.0141	-0.0024	-0.0064	-0.0074	**-0.0402**	0.0132	0.0114	-0.0111	0.0125	0.0135	-0.172
TP	0.0640	-0.0673	-0.1730	-0.0086	-0.2141	-0.0437	-0.0775	-0.0015	0.0 816	**-0.2490**	-0.2843	0.2866	-0.2469	-0.2437	0.282
TF	0.0600	-0.0664	-0.2059	-0.0193	-0.2400	-0.0576	-0.0923	-0.0263	0.0823	-0.2855	**-0.2911**	0.2274	-0.2845	-0.2417	0.215
TC	0.0038	-0.0145	-0.0047	-0.0055	-0.0037	-0.0207	-0.0424	-0.0346	-0.0032	0.0093	-0.0105	**-0.0110**	-0.0941	-0.0631	0.246
DFRS	0.0076	-0.0048	-0.0233	0.0025	-0.0286	-0.0020	-0.0072	0.0031	0.0100	-0.0246	-0.0236	0.0245	**0.0437**	-0.0456	0.227
TS	-0.0126	0.0105	0.0373	0.0001	0.0451	0.0060	0.0140	0.0001	-0.0169	0.0493	0.0487	-0.0494	0.0497	**0.0503**	0.234

Residual value = 0.01208

Note**:** **DFG**=Days to 50% germination, **PH**=Plant height (cm), **DFLP**=Days to first leaf picking, **NPP**= Number of pickings per plant, **NLP**= Number of leaves per plant, **LL**=Leaf length (cm), **LW**=Leaf width (cm), **PL**=Petiole length (cm), **CD**=Crop duration, **TP**=Total phenolics (mg GAE/g), **TF**=Total flavonoids (mg QE/g), **TC**=Total carotenoids (mg/100g), **DFRS**=DPPH free radical scavenging (%), **TS**=Total sugar (%), **GLYPH**=Green leaf yield per hectare (q)

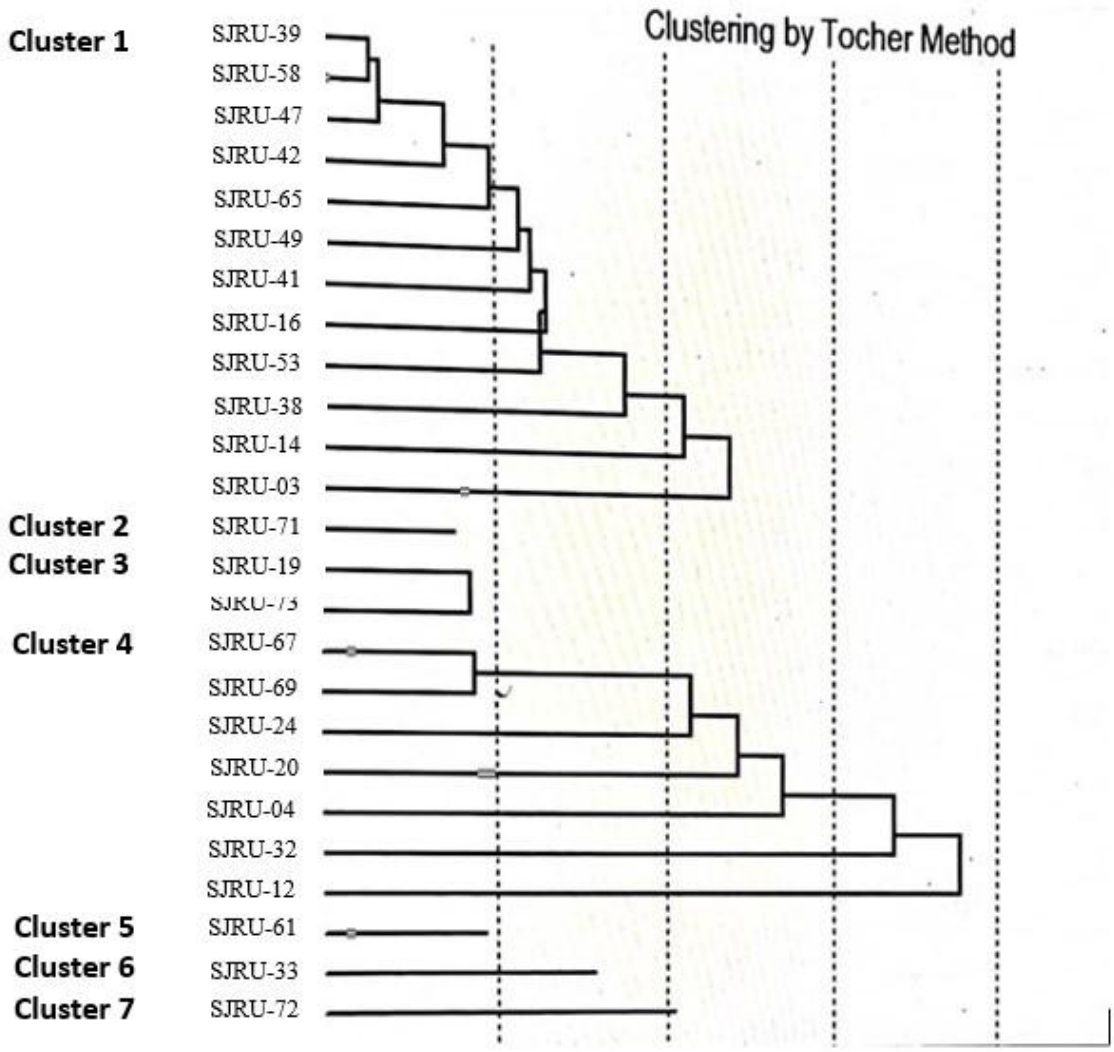


Figure 3: Dendrogram showing clustering pattern of morpho-metrical characters for divergence in *Rumex spp*.

Table 4.8 Clustering of accessions of *Rumex spp.* based on D^2 statistics for morpho-metrical characters

Cluster	Number of accession(s)	Accession (s)
I	12	SJRU-41, SJRU-53, SJRU-16, SJRU-49, SJRU-42, SJRU-47, SJRU-58, SJRU-39, SJRU-65, SJRU-14, SJRU-38 and SJRU-03
II	1	SJRU-71
III	2	SJRU-19 and SJRU-73
IV	7	SJRU-67, SJRU-69, SJRU-24, SJRU-20, SJRU-04, SJRU-32 and SJRU-12
V	1	SJRU-61
VI	1	SJRU-33
VII	1	SJRU-72

Table 4.9 Average intra (bold) and inter cluster (D^2 values) distance values of *Rumex spp.* for morpho-metrical characters.

Cluster	I	II	III	IV	V	VI	VII
I	**13.26**	17.12	18.10	25.00	34.64	40.31	23.97
II		**0.00**	26.66	29.53	33.42	40.09	14.67
III			**9.26**	33.47	46.93	52.52	32.03
IV				**19.19**	22.74	26.45	35.02
V					**0.00**	16.23	40.36
VI						**0.00**	41.62
VII							**0.00**

Table 4.10 Cluster mean values for morpho-metrical characters in *Rumex spp.*

Cluster	DFG	PH	DFLP	NPP	NLP	LL	LW	PL	GLYPP	GLYPH	CD
I	26.56	29.82	75.44	2.36	19.02	16.56	5.84	8.38	1.79	79.57	165.75
II	23.33	30.60	70.67	3.00	23.20	16.15	5.50	7.21	1.64	72.83	161.00
III	33.00	27.71	79.50	2.00	14.90	16.54	5.64	8.42	1.11	49.42	171.17
IV	29.57	29.29	76.43	2.76	21.91	16.41	5.56	7.74	3.00	133.41	166.33
V	23.33	25.60	72.33	3.00	27.73	15.67	5.46	6.15	3.60	160.18	161.00
VI	23.67	38.00	74.33	3.00	28.33	17.40	6.63	8.37	4.00	177.60	161.00
VII	23.33	42.2	78.00	3.00	23.70	16.63	5.87	8.44	1.37	61.10	161.00

DFG=Days to 50% germination, **PH**=Plant height (cm), **DFLP**=Days to first leaf picking, **NPP**= Number of pickings per plant, **NLP**= Number of leaves per plant, **LL**=Leaf length (cm), **LW**=Leaf width (cm), **PL**=Petiole length (cm), **GLYPP=**Green leaf yield (kg)/plot, **GLYPH**= Green leaf yield per hectare (q) and **CD**=Crop duration

Table 4.11 Percentage contribution of morpho-metrical characters towards genetic divergence in *Rumex spp.*

S. No.	Characters	Percentage contribution (%)
1	Days to 50% germination	9.00
2	Plant height (cm)	11.33
3	Days to first leaf picking	0.33
4	Number of pickings per plant	4.67
5	Number of leaves per plant	16.67
6	Leaf length (cm)	0.33
7	Leaf width (cm)	0.33
8	Petiole length (cm)	1.00
9	Green leaf yield (q/ha)	51.34
10	Crop duration	5.00

Table 4.12 Diverse accessions identified for morpho-metrical characters in *Rumex spp.*

S. No.	Characters	Accession(s)
1	Days to 50% germination, days to first leaf picking, number of pickings per plant and crop duration	SJRU-71
2	Days to 50% germination and crop duration, number of pickings per plant	SJRU-61
3	Number of leaves per plant, leaf length, leaf width, green leaf yield/plot and green leaf yield per hectare and crop duration	SJRU-33
4	Days to 50% germination, crop duration, plant height, petiole length and number of pickings per plant	SJRU-72

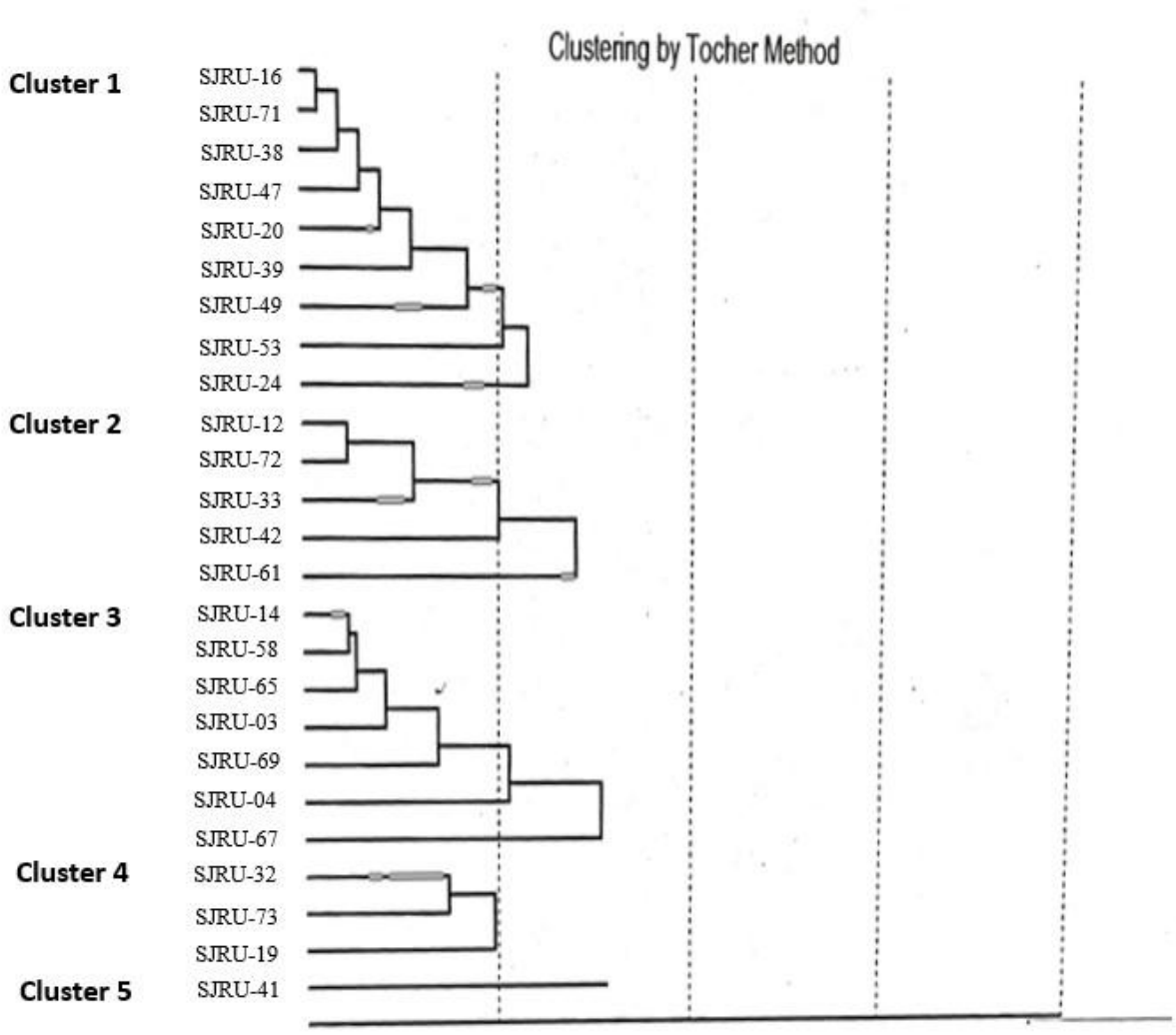


Figure 4: Dendrogram showing clustering pattern of bio-chemical characters for divergence in *Rumex spp*.

SJRU-73. Cluster 4 had 7 accessions namely, SJRU-67, SJRU-69, SJRU-24, SJRU-20, SJRU-04, SJRU-32 and SJRU-12. Cluster 5 had only 1 accession namely SJRU-61. Cluster 6 had only 1 accession namely SJRU-33. Cluster 7 had only 1 accession namely SJRU-72.

4.6.1.2 Inter and intra-cluster distance

Maximum inter-cluster distance was found between cluster 3 and cluster 6 (52.52) followed by cluster 3 and cluster 5 (46.93), whereas minimum inter-cluster distance was found between cluster 2 and cluster 7 (14.67) indicating a close relationship among these accessions.

The maximum intra-cluster distance was found in cluster 4 (19.19) followed by cluster 1 (13.26), indicating an ample amount of genetic divergence within a cluster. Clusters 2, 5, 6 and 7 were with single accession only.

4.6.1.3 Cluster mean analysis

The cluster mean of 25 accessions of rumex was computed in 7 clusters for 11 different characters. The accession in cluster 2 (SJRU-71) had minimum cluster mean value for days to 50% germination, days to first leaf picking and crop duration, whereas it had maximum cluster mean value for number of pickings per plant. The accession in cluster 5 (SJRU-61) had minimum cluster mean value for days to 50% germination and crop duration, whereas it had maximum cluster mean value for number of pickings per plant. The accession in cluster 6 (SJRU-33) had maximum cluster mean value for number of leaves per plant, leaf length, leaf width, green leaf yield/plot and green leaf yield per hectare, whereas it also had minimum cluster mean value for crop duration. The accession in cluster 7 (SJRU-72) had minimum cluster mean value for days to 50% germination and crop duration, whereas it had maximum cluster mean value for plant height, petiole length and number of pickings per plant.

4.6.1.4 Percent contribution towards total divergence

It was observed that green leaf yield contributed maximum (51.67%) followed by number of leaves per plant (16.67%), plant height (11.33%), days to 50% germination (9.00%), crop duration (5.00%), number of pickings per plant (4.67%), petiole length (1.00%), leaf length (0.33%), leaf width (0.33%) and days to first leaf picking (0.33%) towards total divergence.

4.6.2 D^2 statistics for bio-chemical characters

The data analysed for genetic diversity using D^2 statistics for bio-chemical characters is presented in Tables 4.13, 4.14, 4.15, 4.16 and 4.17.

4.6.2.1 Clustering of accessions into different clusters

The genetic diversity among 25 accessions was measured by using D^2 statistics. Based on D^2statistics values, 25 accessions of rumex were grouped into five cluster using Tochers method (Rao,1952) as shown in the dendrogram in Figure 4. Cluster 1 had the highest number of accessions i.e. 9, namely SJRU-16, SJRU-71, SJRU-38, SJRU-47, SJRU-20, SJRU-39, SJRU-49, SJRU-53 and SJRU-24. Cluster 2 had 5 number of accessions namely, SJRU-12, SJRU-72, SJRU-33, SJRU-42 and SJRU-61. Cluster 3 had 7 number of accessions namely SJRU-14, SJRU-58, SJRU-65, SJRU-03, SJRU-69, SJRU-04 and SJRU-67. Cluster 4 had 3 number of accessions namely, SJRU-32, SJRU-73 and SJRU-19. Cluster 5 had only 1 accession namely SJRU-41.

4.6.2.2 Inter and intra-cluster distance

Maximum inter-cluster distance was found between cluster 2 and cluster 4 (8.74) followed by cluster 2 and cluster 5 (8.66), whereas minimum inter-cluster distance was found between cluster 4 and cluster 5 (4.26) indicating a close relationship among the accessions.

Maximum intra-cluster distance was found in cluster 2 (2.55) followed by cluster 4 (2.48), indicating an ample amount of genetic divergence within a cluster. Cluster 5 was with a single accession only.

4.6.2.3 Cluster mean analysis

The cluster mean of 25 accessions of rumex was computed in 5 clusters for 5 different characters. The accessions in cluster 2 (SJRU-12, SJRU-72, SJRU-33, SJRU-42 and SJRU-61) had maximum cluster mean value for total phenolics, total flavonoids, total carotenoids, DPPH free radical scavenging and total sugars.

4.6.2.4 Percent contribution towards total divergence

It was observed that total phenolics contributed maximum (52.00%) followed by total carotenoids (30.33%), total sugars (10.00%), total flavonoids (6.67) and DPPH free radical scavenging (1%) towards total divergence.

Table 4.13 Clustering of accessions of *Rumex spp.* based on D^2 statistics for bio-chemical characters.

Cluster	Number of accession (s)	Accession (s)
I	9	SJRU-16, SJRU-71, SJRU-38, SJRU-47, SJRU-20, SJRU-39, SJRU-49, SJRU-53 and SJRU-24
II	5	SJRU-12, SJRU-72, SJRU-33, SJRU-42 and SJRU-61
III	7	SJRU-14, SJRU-58, SJRU-65, SJRU-03, SJRU-69, SJRU-04 and SJRU-67
IV	3	SJRU-32, SJRU-73 and SJRU-19
V	1	SJRU-41

Table 4.14 Average intra (bold) and inter cluster (D^2 values) distance values of *Rumex spp.* for bio-chemical characters.

Cluster	I	II	III	IV	V
I	**2.10**	4.97	4.69	5.51	5.38
II		**2.55**	6.49	8.74	8.66
III			**2.35**	4.39	4.30
IV				**2.48**	4.26
V					**0.00**

Table 4.15 Cluster mean values for bio-chemical characters in *Rumex spp.*

Cluster	TP	TF	TC	DFRS	TS
I	39.60	9.17	7.10	88.98	8.92
II	44.40	9.91	8.54	94.73	10.01
III	34.16	7.81	8.12	83.32	7.97
IV	31.47	7.22	6.59	80.19	7.69
V	33.76	7.32	6.88	82.12	7.08

TP=Total phenolics, **TF=**Total flavonoids, **TC=**Total carotenoids, **DFRS=**DPPH free radical scavenging **and TS=**Total sugars

Table 4.16 Percentage contribution of bio-chemical characters towards genetic divergence in *Rumex spp.*

S. No.	Characters	Percentage contribution (%)
1	Total phenolics (mg/GA)	52.00
2	Total flavonoids (mg/QE)	6.67
3	Total carotenoids (mg/100g)	30.33
4	DPPH free radical scavenging (%)	1.00
5	Total sugars (%)	10.00

Table 4.17 Diverse accessions identified for bio-chemical characters in *Rumex spp.*

S. No.	Characters	Accession(s)
1	Total phenolics, total flavonoids, total carotenoids, DPPH free radical scavenging and total sugars.	SJRU-12, SJRU-72, SJRU-33, SJRU-42 and SJRU-61

CHAPTER-V

DISCUSSION

Rumex (Sorrel) is an under-utilized vegetable crop rich in bioactive phytochemicals. The present study entitled, entitled **"Variability studies based on morphological and bio-chemical analysis in Rumex (*Rumex spp.*) accessions",** was undertaken to evaluate variability and genetic diversity among the accessions of rumex based on morpho-metrical and bio-chemical characters.

In the present study, twenty-five collections of rumex from various districts of Jammu province were assessed to know the magnitude of genetic variability and heritability of important economic characters, their interrelationships and their direct and indirect effect on yield. The data was obtained regarding nature and extent of variability, heritability, genetic advance and genetic gain to have guidelines for selection of desirable characters. Similarly, correlation coefficients (phenotypic and genotypic) along with path analysis and genetic divergence were worked out for morpho-metrical and bio-chemical characters to select superior accessions. The information gathered shall be effective for planning and execution of improving accessions and development of high-yielding, quality and genetically diverse accessions in rumex. The salient findings obtained on various aspects have been discussed under the following sub-headings:

5.1 Analysis of variance

5.2 Evaluation of accessions

5.3 Parameters of variability

5.4 Genotypic and Phenotypic correlations

5.5 Path coefficient analysis

5.1 Analysis of variance

Analysis of variance revealed mean sum of squares were highly significant for all the characters studied which indicated not only the presence of genetic variability among the accessions but also reflect the extent of diversity in growing conditions. This provides an ample opportunity for selecting suitable accessions with high mean for all the traits of interest. The results are in accordance with the findings of Hiremath *et al.* (2017), Bisht *et al.* (2018), Sharma (2022), Jungova *et al.* (2023) and Papaiahgari *et al.* (2023).

5.2 Evaluation of accessions

In the present case, a considerable amount of variability was observed in all the accessions of rumex as is evident from the analysis of variance. Days to 50 % germination, plant height, number of pickings per plant and days to first leaf picking showed a significant amount of variation among the different accessions under study. Similar results were also reported by Kapila *et al.* (2006), Bisht (2015), Munir *et al.* (2016), Hiremath *et al.* (2017), Mishra *et al.* (2019), Sharma (2022) and Papaiahgari *et al.* (2023). Number of leaves per plant, leaf length, leaf width and petiole length also showed a wide range of variation among the rumex accessions. These results are in conformity with the findings of Kapila *et al.* (2006), Bisht (2015), Munir *et al.* (2016), Mishra *et al.* (2019) and Singh *et al.* (2024). Green leaf yield per plot, yield per hectare and crop duration are the major yield contributing traits and wide range of variability was observed concerning these traits among accessions and these results are in accordance with the findings of Bisht (2015), Munir *et al.* (2016), Bisht *et al.* (2018), Sharma (2022), Papaiahgari *et al.* (2023) and Singh *et al.* (2024). Accessions namely SJRU-33 and SJRU-12 performed better for yield and its contributing characters. The bioactive phytochemicals (such as polyphenols, flavonoids, carotenoids, tocopherols and ascorbic acid) act as an antioxidant and detoxifying agents. Total phenolics and total flavonoids showed a wide range of variation understating the significance of a few accessions having high anti-oxidant, anti-inflammatory and anti-cancerous properties besides their role in food preservation and industrial applications. The results conform with the earlier work of Yildirim *et al.* (2001), Coruh *et al.* (2008), Letowska *et al.* (2009), El-Hawary *et al.* (2011), Mostafa *et al.* (2011), Sahreen *et al.* (2011), El-Bakry *et al.* (2012), Elzaawely *et al.* (2012), Foda *et al.* (2013), Khan *et al.* (2014), Beddou *et al.* (2015), Laouini and Ouahrani (2017), Feduraev *et al.* (2019), Thakur and Baishya (2020) Feduraev *et al.* (2022) and Alkaabi *et al.* (2022). Higher DPPH free radical scavenging activities improve the natural defence mechanism and maintain membrane consistency of vegetables. Wide range of variation was observed for this character and results were similar to the findings of Yildirim *et al.* (2001), Coruh *et al.* (2008), Letowska *et al.* (2009), Harshaw *et al.* (2010), El-Hawary et al. (2011), Mostafa *et al.* (2011), Sahreen *et al.* (2011), El-Bakry *et al.* (2012), Elzaawely *et al.* (2012), Kilic *et al.* (2013), Khan *et al.* (2014), Ahamed *et al.* (2015), Ahamed *et al.* (2016), Laouini and Ouahrani (2017), Feduraev *et al.* (2019), Kengne *et al.* (2021), Hafaz *et al.* (2022) and Salama *et al.* (2022). The total carotenoids and total sugar play an ameliorative role under various abiotic stresses. Park *et al.* (2010), Elzaawely *et al.* (2012) and Beddou *et al.* (2015), also reported variation with respect

to these characters. In the present study, accessions namely SJRU-12, SJRU-33, SJRU-61 for flavonoids, carotenoids and sugars, whereas, SJRU-33, SJRU-12, SJRU-61, SJRU-72 and SJRU-24 for anti-oxidant activities showed maximum values for these compounds. This signifies their role in the food, pharmacological and cosmetics industries. They are not only utilised for nutrition but also for cancer prevention, immune support, gut and eye health etc and possess natural coloring properties with high energy potential. Significant variation in leaf shape type was observed, viz., elliptical (eight accessions), lanceolate (seven accessions), oblanceolate (five accessions), ovate (three accessions), cordate (one accession) and elliptical-cordate (one accession). The spines (in leaf axils) and leaf pigmentation were absent and leaf veins were prominent in all twenty-five accessions of rumex. Based on leaf color, leaves of rumex varied from dull green (twelve accessions), light green (seven accessions) and dark green (six accessions). The current findings are in accordance with the findings of Wang *et al.* (2014), Thapa and Blair (2018) and Sonnberger (2018).

5.2 Parameters of variability

5.2.1 Coefficients of variability

The knowledge of genetic coefficient of variation is necessary for improvement of a crop which helps to measure the extent of genetic variability in the characters among accessions and provides a means to compare the genetic variability present in the various characters. Larger the variability, larger are the chances of selecting desired accessions (Vavilov, 1951).

The estimates of phenotypic and genotypic coefficients of variability give a clear picture of amount of variations present in the available germplasm. In the present study, the phenotypic coefficient of variation (PCV) were higher in magnitude than their corresponding genotypic coefficient of variation (GCV) for all the traits indicating lesser influence of environmental factors, hence selection based on phenotypic value is feasible. Coefficients of variability varied in magnitude from character to character (either low, moderate or high). Therefore, it indicated that there was a great variability in the experimental material used.

In general, estimates of phenotypic coefficient of variation (PCV) were slightly greater than corresponding genotypic coefficient of variation (GCV) indicating the role of environment in the expression of characters. The phenotypic and genotypic coefficient of variability were found to be high for green leaf yield per plot and per hectare. The phenotypic coefficient of variability (PCV) and genotypic coefficient of variability (GCV) were found to be moderate for number of pickings per plant, number of leaves per plant, days to 50% germination, petiole

length, plant height, total phenolics, total flavonoids, total sugar and total carotenoids. Low phenotypic coefficient and genotypic coefficient of variability were observed for crop duration, days to first leaf picking, leaf length, DPPH free radical scavenging and leaf width. The results are in conformity with earlier work of Kapila *et al.* (2006), Bisht (2015), Munir *et al.* (2016), Bisht *et al.* (2018), Hiremath *et al.* (2017), Sharma (2022), Papaiahgari *et al.* (2023) and Singh *et al.* (2024).

5.2.2 Heritability

It is transmissibility of characters from parents to offspring. Heritability in broad sense is the ratio of genotypic variance to the total phenotypic variance and is of primary use when both the additive and non-additive genetic variations can be transferred. The heritability estimates help the plant breeder in selection based on the phenotypic performance but this selection is misleading as Johnson *et al*, (1955) reported that heritability estimates along with expected genetic advance is more useful than heritability alone for improving a particular trait.

High heritability was observed for green leaf yield per plot and yield per hectare, number of leaves per plant, total phenolics, total flavonoids, plant height, total carotenoids, total sugar, days to 50% germination, number of pickings per plant, crop duration, petiole length, DPPH free radical scavenging, leaf width and days to first leaf picking. Moderate heritability was observed for leaf length. The current results are consistent with the findings of Sharma (2022), Hiremath *et al.* (2017) and Papaiahgari *et al.* (2023).

5.2.3 Genetic Advance

Genetic advance (GA) is the improvement over the base population that can potentially be made from selection for a characteristic. It is the function of the heritability of the traits, the amount of phenotypic variation and the selection differential(s) that the breeder uses. High genetic gain as percentage of mean were observed for green leaf yield per plot and per hectare, number of leaves per plant, number of pickings per plant, days to 50% germination, plant height, petiole length, total phenolics, total flavonoids, total sugar and total carotenoids. High heritability along with high genetic advance indicates that these characters are largely controlled by additive gene action, therefore improvement in these characters is possible through selection. Moderate genetic gain was observed for leaf width and DPPH free radical scavenging. High heritability coupled with moderate genetic advance suggested the role of both additive and non-additive gene effects in their inheritance, therefore adoption of breeding programme which could exploit both the gene actions would be a prospective approach. Low

genetic gain was observed for crop duration, days to first leaf picking and leaf length. High heritability coupled with low genetic advance indicated the role of non-additive genes in the inheritance of these characters, hence improvement of these characters through heterosis breeding rather than selection could be adopted. The current results are in conformity with the findings of Sharma (2022), Hiremath *et al.* (2017) and Papaiahgari *et al.* (2023).

5.3 Correlation studies

Selection based primarily on yield which is polygenically controlled character is not that much effective due to the fact that direct improvement for yield is difficult. A knowledge of degree of association of yield with its components is of great importance. It provides information about the nature, extent and direction of selection pressure to be applied for particular consideration. Yield is not an independent character but it is the resultant of the interactions of a number of component characters among themselves as well as with the environment in which the plant grows. Each character is likely to be modified by action of genes present in the genotypes of plant and also by the environment so it becomes difficult to evaluate this complex character directly. Therefore, correlation study of yield with its component traits has been executed, to find out the yield contributing traits. The correlation coefficients among different characters were worked out at phenotypic and genotypic levels. In general, the genotypic correlation coefficients were higher in magnitude than the corresponding phenotypic correlation coefficients.

Days to 50% germination was negatively and significantly correlated with plant height, whereas, it was positively and significantly correlated with days to first leaf picking and crop duration at both phenotypic and genotypic levels but was negatively and significantly correlated with number of leaves per plant, total phenolics and total sugars at phenotypic level only. Plant height was negatively and significantly correlated with crop duration at both phenotypic and genotypic levels, but it was positively and significantly correlated with leaf length and total phenolics at phenotypic level only. Days to first leaf picking was positively and significantly correlated with crop duration at both phenotypic and genotypic levels. Number of pickings per plant was positively and significantly correlated with number of leaves per plant and green leaf yield per hectare at both phenotypic and genotypic levels. Number of leaves per plant was negatively and significantly correlated with crop duration at phenotypic level only, whereas it was positively and significantly correlated with green leaf yield per hectare at both phenotypic and genotypic levels. Leaf length was positively and significantly correlated with leaf width and petiole length at both phenotypic and genotypic levels. Leaf

width was positively and significantly correlated with petiole length at both phenotypic and genotypic levels, but was positively and significantly correlated with total phenolics, total flavonoids, DPPH free radical scavenging and total sugars at phenotypic level only. Crop duration was negatively and significantly correlated with total phenolics, total flavonoids, total carotenoids, DPPH free radical scavenging and total sugars at the phenotypic level only. Total phenolics were positively and significantly correlated with total flavonoids, DPPH free radical scavenging, total sugars and total carotenoids at both phenotypic and genotypic levels. Total flavonoids were positively and significantly correlated with DPPH free radical scavenging and total sugars at both phenotypic and genotypic levels, but was positively and significantly correlated with total carotenoids at phenotypic level only. Total carotenoids was positively and significantly correlated with DPPH free radical scavenging and total sugars at both phenotypic and genotypic levels. DPPH free radical scavenging was positively and significantly correlated with total sugars at both phenotypic and genotypic levels. The findings are in agreement with the early work of Yildirim *et al.* (2001), Sahreen *et al.* (2011), Elzaawely *et al.* (2012), Foda *et al.* (2013), Bisht (2015), Feduraev *et al.* (2022), Sharma (2022) and Papaiahgari *et al.* (2023).

5.4 Path Coefficient Analysis

Although correlation studies are helpful in determining the components of yield but it does not provide a clear picture of nature and extent of contributions made by number of independent traits. In a biological system, the associations between variables may exist in a very complex form. It is therefore essential to study the relationship among variables in a comprehensive way. In order to find a clear picture of the inter-relationship between yield and yield contributing characters, direct and indirect effects were worked out using path analysis. Path coefficient analysis devised by Dewey and Lu (1959) provides a realistic basis for the allocation of appropriate weightage to various attributes for designing a pragmatic programme for the improvement of yield. Path coefficient analysis depicts the effects of different independent traits individually and in combination with other traits on the expression of different traits on marketable fruit yield per plant.

Genotypic path analysis revealed that the maximum direct effect on green leaf yield per hectare was exerted by number of leaves per plant followed by number of pickings per plant, leaf length, total sugar, leaf width, DPPH free radical scavenging and plant height while maximum negative direct effect was shown by total flavonoids followed by total phenolics, petiole length, days to 50% germination, crop duration, days to first leaf picking and total carotenoids. Maximum positive indirect towards green leaf

yield per plot and per hectare was shown by leaf length via number of leaves per plant, followed by leaf width via number of leaves per plant, whereas maximum negative indirect effect was shown DPPH free radical scavenging via total flavonoids followed by total flavonoids via total phenol. Similar results were obtained by Bisht *et al.* (2018), Sharma (2022) and Papaiahgari *et al.* (2023).

5.6 Genetic divergence studies

Genetic divergence is important in determining the actual distance between the genotypes chosen as parents. Hybridization of more divergent parents is expected to increase heterosis and generate a wide range of variability in segregating generations within a certain limit. Based on D^2 values, the clustering pattern of 25 accessions indicated sufficient genetic diversity for selecting superior and diverse parents that can be used in rumex breeding programme. Based on performance for various characters, all the accessions were grouped into 7 diverse clusters for morpho-metrical characters and 5 diverse clusters for bio-chemical characters respectively.

The perusal of data for morpho-metrical characters depicted that cluster I had the maximum number of accessions (12) followed by cluster IV (7), cluster III (2), cluster II (1), cluster V (1), VI (1) and cluster VII (1). The seven resulting clusters for morphological characters had sufficient genetic diversity.

The perusal of data for bio-chemical characters depicted that cluster I had the maximum number of accessions (9) followed by cluster III (7), cluster II (5), cluster IV (3) and cluster V (1). The five resulting clusters for bio-chemical characters had sufficient genetic diversity. Similar results have been reported by Kapila *et al.* (2006), Bisht (2015), Sharma (2022) and Chang *et al.* (2023).

5.6.1 Inter and intra-cluster distance

High heterosis can be exerted by parents who are genetically distant (Ramanujam, *et al*., 1974 and Farhad *et al*., 2010). In the current investigation for morphological characters, the relative inter cluster distance showed maximum divergence between cluster III and VI (52.52) followed by cluster III and V (46.93), suggesting that crossing between the accessions of these clusters will result in more heterosis. The lowest inter cluster distance was observed between cluster II and VII (14.67) followed by cluster V and VI (16.23), suggesting a close relationship between these clusters. Cluster IV had the highest intra-cluster distance i.e. 19.19 followed by cluster I (13.26), indicating sufficient genetic divergence among the accessions of these

clusters. This implies that crossing among the accession of the cluster with the highest intra-cluster value (cluster IV) will result in the greatest amount of heterosis.

In case of bio-chemical characters, the relative inter-cluster distance showed maximum divergence between clusters II and IV (8.74) followed by clusters II and V (8.66), suggesting that crossing between the accessions of these clusters will result in more heterosis. The lowest inter-cluster distance was observed between clusters IV and V (4.26) followed by clusters III and V (4.30), suggesting a close relationship between these clusters. Cluster II had the highest intra-cluster distance i.e. 2.55 followed by cluster IV (2.48), indicating sufficient genetic divergence among the accessions of these clusters. This implies that crossing among the accession of the cluster with the highest intra-cluster value (cluster II) will result in the greatest amount of heterosis. For the hybridization programme, the parents from within cluster can be chosen. Accessions from clusters separated by genetic distance can be used in a hybridization programme to obtain a broad range of variation among segregants. Bisht (2015), Chang *et al.* (2023) and Singh *et al.* (2024) had also reported the similar results.

5.6.2 Cluster mean analysis

The mean values obtained for varying numbers of accessions in each cluster cannot be compared statistically but they are compared to get a relative idea of diversity among the clusters. It is possible to identify the characters influencing the divergence based on the range of means for each character. In the present investigation for morphological characters, accession in cluster II had minimum cluster mean value for days to 50% germination, days to first leaf picking and crop duration, whereas it had maximum cluster mean value for number of pickings per plant. Cluster V had minimum cluster mean value for days to 50% germination and crop duration, whereas it had maximum cluster mean value for number of pickings per plant. Cluster VI had maximum cluster mean value for number of leaves per plant, leaf length, leaf width, green leaf yield/plot and green leaf yield per hectare, whereas it also had minimum cluster mean value for crop duration. Cluster VII had minimum cluster mean value for days to 50% germination and crop duration, whereas it had maximum cluster mean value for plant height, petiole length and number of pickings per plant.

In case of bio-chemical characters, the accessions in cluster II had maximum cluster mean value for total phenolics, total flavonoids, total carotenoids, DPPH free radical scavenging and total sugars. A similar comparison of clusters based on the range of mean value

of each character has been done by earlier workers namely, Bisht (2015) and Singh *et al.* (2024).

5.6.3 Percent contribution of various characters

The selection and choice of parents are primarily based on the contribution of traits to divergence. Green leaf yield/plot followed by number of leaves per plant, plant height, days to 50% germination, crop duration, number of pickings per plant, petiole length, leaf length and leaf width contributed the most to genetic divergence for morphological characters.

In case of bio-chemical characters, total phenolics followed by total carotenoids, total sugars, total flavonoids and DPPH free radical scavenging contributed the most towards genetic divergence. While considering the genetic diversity of the parents to be included in the hybridization programme, their field potential should not be overlooked (Rohit *et al*., 2011). Similar results have been obtained by Sharma (2022) and Chang *et al.* (2023).

CHAPTER-VI

SUMMARY AND CONCLUSIONS

The present investigation entitled, **"Variability studies based on morphological and bio-chemical analysis in Rumex (*Rumex spp.*) accessions"** was conducted at Vegetable Experimental Farm-I, Division of Vegetable Science, Faculty of Horticulture and Forestry, Sher-e-Kashmir University of Agricultural Sciences and Technology, Main Campus, Chatha, Jammu (J&K) during winter season 2023. The experiment was conducted in Randomized Complete Block Design with three replications. Data was collected on various characters viz., days to 50 % germination, plant height, days to first leaf picking, number of pickings per plant, number of leaves per plant, leaf length, leaf width, petiole length, green leaf yield per plot and per hectare, crop duration, total phenolics, total flavonoids, total carotenoids, DPPH free radical scavenging, total sugars, leaf shape type, prominence of leaf veins, spines in leaf axils, leaf pigmentation and leaf color.

The ANOVA indicated the existence of substantial differences amongst accessions studied. SJRU-53 & SJRU-65 were found to be the earliest for days to 50 % germination while SJRU-72 showed maximum plant height. The maximum number of pickings per plant were recorded in 13 accessions (SJRU-04, SJRU-12, SJRU-20, SJRU-24, SJRU-33, SJRU-39, SJRU-47, SJRU-58, SJRU-61, SJRU-65, SJRU-67, SJRU-71 & SJRU-72). The maximum number of leaves were recorded in accession SJRU-33 (28.33). Accession SJRU-39 showed maximum leaf length, leaf width and petiole length. The maximum green leaf yield per plot & per hectare was recorded in accession SJRU-33. The earliest days for crop duration were recorded in six accessions viz., SJRU-16, SJRU-33, SJRU-53, SJRU-61, SJRU-71 and SJRU-72.

The maximum total phenol content and DPPH free radical scavenging percentage were recorded in the accession SJRU-33. The maximum total flavonoids were observed in accession SJRU-12. The maximum total carotenoid and total sugar content was observed in accession SJRU-61.

The leaves of rumex showed variability in leaf shape type as 6 types of leaves were observed, viz., elliptical (eight accessions), lanceolate (seven accessions), oblanceolate (five accessions), ovate (three accessions), cordate (one accession) and elliptical-cordate (one accession). The spines (in leaf axils) and leaf pigmentation were absent and leaf veins were prominent in all twenty-five accessions of rumex. Based on leaf color, leaves of rumex varied

from dull green (twelve accessions), light green (seven accessions) and dark green (six accessions).

The estimates of the phenotypic coefficients of variability were greater than the corresponding genotypic coefficients of variability for all the characters investigated. This indicated that the environment had an impact on these characters. The phenotypic and genotypic coefficient of variability was found to be high for green leaf yield per plot and per hectare.

High heritability estimates were observed for characters namely, green leaf yield (kg)/plot, yield per hectare, number of leaves per plant, total phenolics, total flavonoids, plant height, total carotenoids, total sugar, days to 50% germination, number of pickings per plant, crop duration, petiole length, DPPH free radical scavenging, leaf width and days to first leaf picking. This acknowledges that selection for characters with high heritability can be done quickly. High heritability with high genetic advance was observed in green leaf yield and number of leaves per plant.

A significant and positive correlation was observed for number of pickings per plant and number of leaves per plant with green leaf yield per hectare. Significant and positive correlation was observed for total flavonoids, DPPH free radical scavenging, total sugars and total carotenoids with total phenolics.

Path coefficient analysis indicated that the maximum positive direct effect towards green leaf yield per hectare was contributed by number of leaves per plant followed by number of pickings per plant, whereas the maximum positive indirect effect was shown by leaf length via number of leaves per plant, followed by leaf width via number of leaves per plant.

Using D^2 statistics, the genetic diversity among the available germplasm was assessed, and 25 accessions were grouped into 7 and 5 diverse clusters for morpho-metrical and bio-chemical characters, respectively.

In case of morpho-metrical characters, cluster I had the maximum number of accessions (12) followed by cluster IV (7), cluster III (2), Cluster II, V, VI and VII comprised of 1 accession only.

The relative inter cluster distance revealed maximum divergence between cluster III and cluster VI, having sufficient genetic divergence among the advanced breeding lines of the cluster. The maximum intra cluster distance was recorded in cluster IV. This demonstrated that the parents for the hybridization programme could be chosen from within the cluster.

Analysis of cluster mean value indicated that accession in cluster II had minimum cluster mean value for days to 50% germination, days to first leaf picking and crop duration, whereas it had maximum cluster mean value for Number of pickings per plant. Cluster V had minimum cluster mean value for days to 50% germination and crop duration, whereas it had maximum cluster mean value for number of pickings per plant. Cluster VI had maximum cluster mean value for number of leaves per plant, leaf length, leaf width, green leaf yield/plot and green leaf yield per hectare, whereas it also had minimum cluster mean value for crop duration. Cluster VII had minimum cluster mean value for days to 50% germination and crop duration, whereas it had maximum cluster mean value for plant height, petiole length and number of pickings per plant.

Green leaf yield contributed maximum towards divergence for morpho-metrical characters.

Amongst clusters of bio-chemical characters, cluster I had the maximum number of accessions (9) followed by cluster III (7), cluster II (5), cluster IV (3) and cluster V (1).

The relative inter-cluster distance revealed maximum divergence between cluster II and cluster IV, having sufficient genetic divergence among the advanced breeding lines of the cluster. The maximum intra-cluster distance was recorded in cluster II. This demonstrated that the parents for the hybridization programme could be chosen from within these clusters.

Analysis of cluster mean value indicated that the accessions in cluster II had maximum cluster mean value for total phenolics, total flavonoids, total carotenoids, DPPH free radical scavenging and total sugars.

Total phenolics contributed the most towards genetic divergence for bio-chemical characters.

Based on the findings of this investigation, it can be concluded that:

- Mean performance of accessions for morpho-metrical characters revealed that two accessions viz. SJRU-33 and SJRU-12 performed better than all other accessions, whereas, SJRU-12, SJRU-72, SJRU-33, SJRU-24 and SJRU-61 performed better than all other accessions for most of the bio-chemical characters. Accessions SJRU-12, SJRU-61 and SJRU-72 were lanceolate whereas, SJRU-33 was cordate and SJRU-24 was oblanceolate in shape. SJRU-12, SJRU-33 and SJRU-72 were dark green, SJRU-24 was dull green and SJRU-61 was light green in color. All of these had prominent

leaf veins but lacked leaf pigmentation and spines (in leaf axils). These accessions could be utilized for valuable food source, natural food preservatives, skin care products and can inform breeding programs leading to improved crop varieties.

- Genetic analysis indicated that phenotypic coefficient of variation (PCV) was in general higher in magnitude than the corresponding genotypic coefficient of variation (GCV) for all characters studied, which signified the presence of environmental influence to some degree in the phenotypic expression of characters. The highest PCV and GCV were obtained for green leaf yield per plot and per hectare.
- High heritability coupled with high genetic gain was obtained for green leaf yield per hectare and number of leaves per plant. Hence, the selection of these characters for improvement could be more successful and efficient.
- Green leaf yield per hectare had positive and significant correlation with number of pickings per plant and number of leaves per plant. Therefore, direct selection for these characters can be used for rumex yield improvement. On the other hand, total phenolics had positive and significant correlation with total flavonoids, DPPH free radical scavenging, total sugars and total carotenoids. Therefore, direct selection for these characters can be used for rumex bio-chemical characterisation.
- The number of leaves per plant showed the greatest positive direct effect on green leaf yield per plot and per hectare, followed by number of pickings per plant showing their suitability for direct selection.
- Based on D^2 analysis, 25 accessions of rumex were grouped into 7 diverse clusters for morpho-metrical characters and 5 diverse clusters for bio-chemical characters respectively. In case of morpho-metrical clusters, cluster I had the maximum number of accessions (12), followed by cluster IV (7), cluster III (2), Cluster II, V, VI and VII comprised of only 1 accession only. In, bio-chemical clusters, cluster I had the maximum number of accessions (9) followed by cluster III (7), cluster II (5), cluster IV (3) and cluster V (1). The maximum inter cluster distance was observed between cluster III and cluster VI for morpho-metrical characters and between cluster II and cluster IV for bio-chemical characters. Thus, it can be concluded that there is sufficient diversity among different accessions for various parameters which can be exploited for producing elite hybrids or segregants in the subsequent generations.

CHAPTER-VII

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APPENDIX – I

Various weekly weather parameters recorded during crop growing period (*Rabi*-2023-24)

SMW	Date and month	Max. Temp. (°C)	Min. Temp. (°C)	Morning RH (%)	Evening RH (%)	Rainfall (mm)
49	30-06 Dec	23.8	7.2	96	47	0.9
50	7-13 Dec	21.3	4.8	96	49	0.9
51	14-20 Dec	21.8	4.6	95	49	1.5
52	21-27 Dec	18.0	6.4	96	70	1.3
1	4-10 Jan	11.5	6.0	94.3	83.6	0.0
2	11-17 Jan	9.1	4.4	97.0	88.6	0.0
3	18-24 Jan	13.7	3.3	96.6	71.6	0.0
4	25-31 Jan	15.5	4.9	95.3	64.7	0
5	1-7 Feb	17.5	7.2	92.4	67.7	28.8
6	8-14 Feb	21.1	2.8	94.7	45.1	2.6
7	15-21 Feb	22.8	5.9	93.9	51.1	0.0
8	22-28 Feb	21.4	6.4	90.6	46.7	10.4
9	29-6 Mar	21.2	7.8	90.6	52.4	57.4
10	7-13 Mar	23.8	7.2	92.1	42.9	0.0
11	14-20 Mar	26.3	9.0	90.9	40.7	2.6
12	21-27 Mar	29.2	13.2	83.6	43.9	0.0
13	28-3Apr	29.4	14.9	86.4	62.9	12.6
14	4-10Apr	32.5	12.3	81	42	0
15	11-17Apr	32.2	16.2	82.4	41.4	6.6
16	18-24 Apr	33.8	16.0	79.71	40.71	2.25
17	25-01 May	36.0	12.0	70.57	36.57	3.94
18	02-08 May	39.0	14.0	67.85	30.71	0.0
19	09-15 May	39.4	15.6	70.71	35.28	0.57
20	16-22 May	42.0	21.5	56.28	26.71	0.0
21	22-28 May	42.4	20.4	62.28	31.85	0.40

Source: Agro meteorology observatory, SKUAST- J, Chatha

APPENDIX – II

Analysis of Variance for various characters in 25 accessions of *Rumex spp.* L. accessions

Characters	Mean Sum of Squares		
	Degree of Freedom		
	Replication (2)	Accessions (24)	Error (48)
Days to 50% germination	1.2100	63.15**	1.213
Plant height (cm)	0.2500	66.56**	0.918
Days to first leaf picking	1.6100	28.86**	4.474
Number of pickings/plant	0.0100	0.72**	0.027
Number of leaves/plant	0.0100	41.56**	0.213
Leaf length (cm)	0.1200	2.39**	0.560
Leaf width (cm)	0.3200	0.8**	0.101
Petiole length (cm)	0.67	4.58**	0.297
Green leaf yield(kg)/plot	0.0200	2.19**	0.009
Green leaf yield per hectare(q)	31.1200	4324.56**	17.879
Crop duration	2.1700	45.87**	2.187
Total phenolics (mg GAE/g)	0.6500	67.05**	3.459
Total flavonoids (mg QE/g)	0.0300	3.34**	0.170
Total carotenoids (mg/100g)	0.0400	1.8**	0.202
DPPH free radical scavenging (%)	4.3900	82.93**	7.815
Total sugars (%)	0.0500	2.59**	0.140

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CERTIFICATE- IV

Certified that all the necessary corrections as suggested by the external examiner and advisory committee have been duly incorporated in the thesis entitled **"Variability studies based on morphological and bio-chemical analysis in Rumex (*Rumex spp.*) accessions"**, submitted by **Mr. Sunit Jarngal,** Registration No. **J-22-MHF-21**.

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