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Graphical Evaluation of Genetic Diversity among Some Genotypes of *Lepidium sativum* L. Using Morphological Traits

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Abstract

A two-year study of garden cress (*Lepidium sativum* L.) was conducted, evaluating 81 foreign (from the Genebank Department of Leibniz Institute of Plant Genetics and Crop Plant Research (IPK; Institut für Pflanzengenetik und Kulturpflanzenforschung) in Gatersleben, Germany) and Iranian (local cultivars) genotypes for seed yield and 18 traits. The first two principal components of the genotype \times trait interaction biplot accounted for 70% and 61% of the total genotype \times trait interaction for the years 2012 and 2013, respectively. The vertex genotypes for the first year were G23, G59, G61, G51, G6, and G12 and for the second year were G32, G78, G53, and G20. Traits, such as seed yield and biological yield, were favorable in sectors of G32 and G12 and these vertex genotypes fell in the same sectors in both years. A positive association was observed between plant height and biological yield, as well as between the main stem axis and seed yield in both years as indicated by the acute angles. Based on seed yield, Genotype G6 was the best followed by Genotypes G29, G32, G48, and G50 in the first year and Genotype G32 followed by Genotypes G29 and G42 were the best genotypes in the second year. Given the importance of main axis length, biological yield, plant height, and flowering period regarding seed yield in this and previous investigations, these traits should be selected in garden cress.

Keywords: Biplot Analysis, Germplasm Collection, Genetic Variation.

Introduction

Garden cress (*Lepidium sativum* L.) is a plant belonging to the Brassicaceae family. It is native to South West Asia and has spread to various regions worldwide. Several floristic investigations in Iran have reported the existence of this species (Sanandaji & Mozaffarian, 2010; Eshghi-Malayeri et al., 2015; Dehshiri, 2016). While both the seeds and leaves of garden cress are beneficial, the crop is primarily cultivated for its seeds, which are an important food source often consumed as a leaf vegetable (Ramadan & Oraby, 2020). It can be harvested throughout the year, whether grown indoors or outdoors. It thrives in fertile, well-worked soil with suitable drainage (Tadesse et al., 2018). Garden cress exhibits significant morphological diversity due to selection although breeding efforts have been somewhat limited (Roughani et al., 2018). Understanding the extent of this variation is crucial for its future breeding and efficient utilization of its genetic resources (Arabsalehi et al., 2016; Fathi et al., 2019). The limited existing literature on garden cress suggests that there is substantial genetic variation in morphological characters (Bhalala & Parmar, 2019). Bansal et al. (2012) estimated the genetic diversity of 18 garden cress genotypes using RAPD molecular markers and divided them into two main

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clusters. Bedassa et al. (2013b) grouped some genotypes into 7 clusters based on morphological characters and geographical properties. It is evident that determining its available variation in plant patterns and yield components is of great importance.

In some investigations, it has been found that among yield components, the thousand-seed weight, number of seeds per silique, and number of siliques per plant are positively correlated with seed yield (Rahi et al., 2012). Bedassa et al. (2013a) reported associations between harvest index, seed yield per plant, biomass yield per plant, and biomass yield per plot using 43 genotypes. Currently, garden cress acreage is limited due to its low yields and lack of stability in registered cultivars. Consequently, the relationships among seed yield, yield components, and other traits remain contentious topics in research. Tadesse et al. (2018) evaluated the genetic variation of 112 garden cress accessions using microsatellite markers, attributing about 80% and 20% of within-accession and between-accession variations, respectively. Similar studies involving biplot analysis of genetic variation have been conducted by Nayebi-Aghbolag et al. (2022) on rye (*Secale cereale* L.) genotypes and by Faheem et al. (2023) on wheat (*Triticum aestivum* L.). Mohebodini et al. (2018) studied the associations between yield performance and certain morphological traits of garden cress accessions, recommending the thousand-seed weight and number of pods per plant as selection criteria in breeding programs.

The purpose of some genetic improvement projects is to identify the best genotypes based on several traits. Understanding the reasons behind Genotype \times Trait (GT) interaction can help identify the best genotypes for selection based on traits, but it is often challenging to discern the structure of genotypic performance across various traits. Various tools have been employed to explore the reasons for GT interaction and while strategies may differ, different approaches have yielded similar results for a given dataset. The use of a site-regression combined genotype (G) main effect and Genotype \times Environment (GE) interaction, such as a GGE biplot for yield stability analysis, has been proposed. This methodology can also be applied to all types of two-way data, including genotype \times trait structure. Yan and Rajcan (2002) utilized a GT biplot technique to study genotype \times trait data and demonstrated that this method is a valuable tool for visualizing genotype \times trait data. Given the limited knowledge about trait associations of garden cress, the goals of this research were to (i) evaluate the performance of 81 garden cress genotypes, (ii) demonstrate the interrelationship among garden cress traits, and (iii) compare genotypes based on multiple traits by using GGE biplot methodology, which could be beneficial for future breeding programs of garden cress.

Materials and Methods

We examined a collection of 81 genotypes across 18 morphological traits with some key properties detailed in Table 1. 77 genotypes were obtained from IPK, Gatersleben, Germany, while 4 were Iranian accessions. Field experiments were carried out over two growing years (2011-2012 and 2012-2013) in Maragheh, northwestern Iran (37° 23' N; 46° 16' E), on loamy clay soil with a pH of 7.3. Each year, we employed a replicated 9 \times 9 simple lattice design with 4 replications. Each plot consisted of 6 rows, which were 0.30 m apart and 2 m long and had a size of 3.6 m². Planting took place on the first day of May with a plant density of approximately 22 plants per square meter. We assessed the following plant characteristics at 10 random points per plot: height of first branch (HFB), height of first silique (HFS), main axis length (MAL), number of lateral branches (NLB), number of silique per lateral branches (NSL), number of siliques per main axis (NSM), number of siliques per plant (NSP), number of seeds per silique of lateral branches (SLB), number of seeds per silique of main axis (SMA), number of seeds of silique per plant (NSSP), and plant height (PH). Additionally, we recorded days to emergence (DE), emergence percent (EP), days to flowering (DF), flowering period (FP), and days to maturing (DM) as accurately as possible. The number of days to flowering after 50% of plants in each plot opened one flower was also recorded. Furthermore, the thousand-seed weight (TSW) was determined from a sub-sample of seeds harvested from each plot. To calculate biological yield and seed yield, the middle four rows were removed (the harvested area was 1.8 m²). We utilized the GT biplot method (Yan & Rajcan, 2002) to illustrate the genotype by trait two-way data and the graphs were generated using the GGEbiplot software (Yan, 2001).

Research Findings

Significant differences ($p < 0.01$) were observed among years for all traits and accessions also exhibited significant differences ($p < 0.01$) for all traits (data not shown). The GT biplot for each of the two years explained 70% and 61% of the total variation of the standardized data, respectively. This low percentage indicated complexity of association among the measured traits of garden cress. For both years, the biplots showed that most of the measured traits contributed to the largest variation as indicated by the relative length of their vectors (see Figures 1A and 1B). The relationships revealed by the vector-view biplot (Figure 1A) were as follows: a positive association between PH and BY; between MAL and SY; among FP, NSL, NSM, and EP; among DM, NSP, SLB, SMA, and NSP; and among NLB, HFB, DF, HFS, and DE as shown by the acute angles in the first year (Figure 1A). Additionally, a negative association existed for traits of PH and BY versus traits of DM, NSP, SLB, SMA, and NSP and such negative association existed for traits of MAL and SY versus traits of NLB, HFB, DF, HFS, and DE as shown by the large obtuse angles between vectors of these traits in the year 2012 (Figure 1A). Figure 1B showed relatively similar results for the second year, clearly suggesting representations of the performances of the accessions. The relationships revealed by the biplot were as follows: a positive association between PH and BY; among MAL, SY, and FP; between NSM and EP; among SLB, TSW, NSP, and SMA; among NSL, DM, and NSP; between DE and NLB; and among DF, HFB, and HFS as shown by the acute angles in the second year (Figure 1B). Additionally, a negative association existed for traits of PH and BY versus traits of NSL, NSP, and DM; traits of MAL, SY, and FP versus traits of DE and NLB and such negative association existed for traits of NSM and EP versus traits of DF, HFB, and HFS as shown by the large obtuse angles between vectors in the year 2013 (Figure 1B).

Figure 2A depicts the biplot derived from data on 19 morphological traits of 81 garden cress genotypes in the experimental year of 2012. From Figure 2A, the following information can be gleaned: The vertex genotypes for the first year are G23, G59, G61, G51, G6, and G12 and the traits fall into the sectors of G23 and G12 in the first year. Consequently, genotype of G12 exhibits the highest



values for PH, MAL, SY, and BY traits, while genotype of G23 displays the highest values for the remaining traits (Figure 2A). Therefore, in Figure 2A, there are two vertex genotypes that are favorable for some traits, but the remaining four vertex genotypes are not favorable for any garden cress traits. In Figure 2B, there are four vertex genotypes (genotypes of G32, G78, G53, and G20), and three of them (G32, G53, and G20) are favorable for some traits, while one of them (G78) is not favorable for any traits in the second year (Figure 2B). Thus, genotype of G53 exhibits the maximum values for HFB, HFS, and DF traits; genotype of G20 displays the highest values for NSL, DM, NSP, NLB, and DE traits; and genotype of G32 has the highest values for the remaining traits (Figure 2B). However, other traits, such as SY and BY, are profitable in the sectors of G32 and G12 in both growing seasons and these vertex genotypes fall into the same sectors in both years.

A combined ANOVA across two years (results not shown) confirmed the results of the GT biplot, indicating significant genotype by year interactions in most of the measured traits. This suggested that garden cress genotypes might only produce high seed yield in certain years. Therefore, the GT biplot might provide ANOVA information, as well as additional insights (Dehghani et al., 2008). However, genotypes of G12, G20, G23, G32, and G53 as the vertex genotypes were the most favorable genotypes for the most important studied traits of garden cress in both years. Thus, the GT biplot accounted for a relatively large portion of the variation (70% and 61% for the first and second year, respectively). Sabaghnia et al. (2010) reported similar findings in their investigation of the effects of genotype, environment, treatment, trait, and their interactions in rapeseed (*Brassica napus* L.). For the evaluation of plant material based on a single trait that allows ranking of genotypes, the seed yield trait was selected. In the year 2012 (Figure 3A), genotype of G6 emerged as the best genotype followed by genotypes of G29, G32, G48, and G50, all of which exhibited above-average values for seed yield. Genotypes below the perpendicular line yielded below average with genotypes of G59 and G61 being the lowest-yielding genotypes. Based on the experiment in 2013 (Figure 3B), the most favorable genotypes was genotype of G32 followed by genotypes of G29 and G42 and then by genotypes of G6, G25, G26, and G81. The lowest-yielding genotype located below the perpendicular line was genotype of G78 followed by G53 and G77 (Figure 3B).

As seed and biological yield, number of siliques, number of seeds per silique per plant, and 1000-seed weight of garden cress genotypes were major breeding goals, they were evaluated and analyzed in the GT biplot (Figure 4A). In the first year, no interrelationships were found between seed yield and other yield components as demonstrated by the relatively perpendicular vectors (Figure 4A). However, there was a positive correlation between seed yield and biological yield as shown by the acute angle between their vectors and a positive interrelationship between the number of siliques per plant and the number of seeds per silique per plant traits as demonstrated by the acute angle between their vectors (Figure 4A). In the second year, we found relatively similar results, but there was a moderate positive association between the number of seeds per silique per plant and 1000-seed weight traits (Figure 4B). This is in agreement with the findings of Coimbra et al. (2004) and Dehghani et al. (2008), who reported similar results in the evaluation of some rapeseed genotypes. However, there was no positive correlation between seed yield and yield components, such as number of siliques per plant, number of seeds per silique per plant, and 1000-seed weight traits in both years, which might indicate that these characteristics could be modified independently of each other. It seemed that among the above characteristics, biological yield was more noticeable and had a positive correlation with seed yield and could be used as an indirect selection index for breeding seed yield.

We utilized the GT biplot to compare 81 garden cress genotypes based on 19 traits and to identify superior genotypes or groups for targeted trait improvement in the genetic enhancement program of garden cress. Figures 2A and 2B revealed that genotypes of G12, G20, G23, G32, and G53 were the most favorable for most of the studied traits with G12 (*Lepidium sativum* L. var. *latifolium* DC. with unknown origin) and G32 (*Lepidium sativum* L. var. *latifolium* DC. from Georgia) standing out as the most favorable genotypes for seed yield potential. These genotypes consistently demonstrated high seed yield and biological yield across both years. It appeared that the yield potential of var. *latifolium* DC. was superior to that of var. *sativum*. As reported by Bermejo and Leon (1994), *L. sativum* was categorized into 3 types or varieties—*sativum*, *crispum*, and *latifolium*—based on botanical features of the leaf, stem, and root. This plant was classified as a neglected and under-utilized horticultural crop. Given the promising potential of garden cress from various perspectives, it is imperative to initiate a comprehensive breeding program to introduce new and improved garden cress cultivars.

We observed that biological yield, main axis length, flowering period, and plant height were positively correlated with seed yield (Figures 1A and 1B). However, we did not find strong positive interrelationships between seed yield and other yield components, such as number of siliques per plant, number of seeds per silique per plant, and 1000-seed weight. We could report a relatively weak positive correlation or no correlation between seed yield and these yield component traits. In a path analysis of 49 garden cress landraces, both at the phenotypic and genotypic levels, Bedassa et al. (2013a) demonstrated that the number of seeds per plant, days to flowering initiation, biological yield, harvest index, and 1000-seed weight had a strong effect on seed yield. This suggests that breeding programs should focus on improving biological yield and flowering characteristics, while also not neglecting the improvement of the most important yield component traits, such as number of siliques per plant, number of seeds per silique per plant, and 1000-seed weight.

Angelini et al. (1997), in their investigation of new potential oilseed crops for industrial uses, evaluated garden cress as an oilseed crop and reported that the overall mean seed oil content was about 18%, ranging from 11% to 23%. Oleic acid was identified as the main fatty acid, ranging from 29.4% to 35.6%, while linolenic acid was the second most prevalent fatty acid in garden cress with its content varying from 20.4% to 29.5%. Bedassa et al. (2013a) reported a high potential for garden cress seed yield (approximately 2.4 t ha⁻¹) and compared it with the yield of linseed (flax) in Ethiopia, concluding that garden cress will make a significant contribution to increasing edible oil supply in the future. According to Angelini et al. (1997), the seed yield potential of the garden cress germplasm collection in Italy ranged from 0.3 to 2.5 t ha⁻¹. The advantages of garden cress cultivation include its short cycle length and good tolerance to drought. However, its current disadvantages are low seed yield potential, lodging (exceeding 60%), low oil content in the seed, and low levels of a single fatty acid in its seeds. To make garden cress potentially suitable for cultivation, further

genetic improvement work should be undertaken to increase its potential and eliminate certain undesirable characteristics (Angelini et al., 1997).

For garden cress cultivars, two traits are of utmost importance: seed yield and biological yield. The trait vectors displayed in Figures 1A and 1B illustrate the interrelationship among them, which is typical of other datasets (Yan & Rajcan, 2002), revealing that these traits are almost dependent on each other. The trait relationships (Figures 1A and 1B) include zero or near-zero associations between seed yield (SY) and biological yield (BY) versus number of seeds per plant (NSP), number of seeds per silique per plant (NSSP), 1000-seed weight (TSW), number of lateral branches (NLB), number of siliques per lateral branches (NSL), number of siliques per main axis (NSM), number of seeds per silique of lateral branches (SLB), and number of seeds per silique of main axis (SMA) traits. Seed yield and biological yield are crucial for growers and these weak associations suggest that it is difficult to incorporate all three into a single cultivar. However, some of them can be reasonably combined, albeit at the expense of others, presenting a major challenge for garden cress breeding programs. The studied garden cress germplasm indicated its potential for genetic improvement of most characteristics using GT biplots. Therefore, it was easy to identify genotypes with characteristics different from local cultivars for earliness and seed yield, as well as yield components, such as number of siliques per plant, number of seeds per silique per plant, and 1000-seed weight. We found a positive association among main axis length (MAL), biological yield (BY), plant height (PH), and seed yield (SY) traits. Sabaghnia et al. (2010) reported similar results in rapeseed (*Brassica napus* L), and Mohebodini et al. (2018) in garden cress. Additionally, we found a positive association among number of siliques per lateral branches (NSL), number of siliques per main axis (NSM), and 1000-seed weight (TSW) traits. Similar reports have been made by Nayebi-Aghbolag et al. (2022) in rye (*Secale cereale* L.). Furthermore, positive associations were found for height of first silique (HFS), height of first branch (HFB), days to flowering (DF), days to emergence (DE), number of lateral branches (NLB), and days to maturity (DM), which were relatively similar to the results reported by Bedassa et al. (2013a) in garden cress and Yadav et al. (2012) in Indian mustard.

The GGE biplot originally designed for analyzing multi-environment trials is also applicable to all two-way tables, including genotype \times trait datasets. Given that different traits have different units, standardization is necessary before biplot analysis. It is undeniable that a biplot of the first two principal components will be as effective as conventional analysis methods if they account for a suitable amount of variation. Additionally, the biplot provides a graphical representation of genotype \times trait pattern and with the assistance of GGEbiplot, dataset visualization becomes straightforward (Sabaghnia et al., 2012). Ultimately, the biplot offers a comprehensive depiction of the interrelationships among genotypes and traits. For these reasons, we preferred using the GGE biplot model for analyzing garden cress datasets. Similar reports have demonstrated that GT biplots are an excellent tool for visualizing genotype \times trait datasets and revealing the interrelationships among traits (Nayebi-Aghbolag et al., 2022).

In conclusion, the results indicated that garden cress germplasm could serve as a valuable source of genes for seed yield and for enhancing various yield components, such as number of siliques per plant, number of seeds per silique per plant, and 1000-seed weight. This study demonstrated the effectiveness of the GT biplot as a tool for visualizing genotype by trait data. By employing this technique in our garden cress trials, we were able to clearly illustrate the interrelationships among garden cress traits, providing valuable insights into these relationships. Considering the significance of main axis length, biological yield, plant height, and flowering period in relation to seed yield both in this study and in previous research, it is recommended that high-yielding genotypes with high thousand seed weight (TSW) and short days to maturity be selected in garden cress breeding programs.

Figures & Tables

Table 1: Scientific names and origins of 77 *Lepidium sativum* L. accessions from IPK and 4 local Iranian landraces used in this study

Code	Accession	<i>L. sativum</i> L. var.	Origin	Code	Accession	<i>L. sativum</i> L. var.	Origin	Code	Accession	<i>L. sativum</i> L. var.	Origin
G1	LEP-1	sativum	Afghanistan	G28	LEP-40	sativum	Iraq	G55	LEP-73	sativum	Germany
G2	LEP-2	latifolium DC.	Afghanistan	G29	LEP-42	sativum	Iraq	G56	LEP-74	sativum	Yemen
G3	LEP-3	sativum	Greece	G30	LEP-43	latifolium DC.	Georgia	G57	LEP-75	latifolium DC.	Azerbaijan
G4	LEP-4	sativum	unknown	G31	LEP-44	sativum	Georgia	G58	LEP-76	latifolium DC.	Germany
G5	LEP-5	crispum (Medik.) DC.	unknown	G32	LEP-45	latifolium DC.	Georgia	G59	LEP-78	sativum	Germany
G6	LEP-8	sativum	unknown	G33	LEP-46	sativum	Italy	G60	LEP-86	L. sativum	unknown
G7	LEP-9	sativum	unknown	G34	LEP-47	latifolium DC.	Ukraine	G61	LEP-102	L. sativum	unknown



Code	Accession	<i>L. sativum</i> L. var.	Origin	Code	Accession	<i>L. sativum</i> L. var.	Origin	Code	Accession	<i>L. sativum</i> L. var.	Origin
G8	LEP-12	crispum (Medik.) DC.	unknown	G35	LEP-48	sativum	Georgia	G62	LEP-103	sativum	Austria
G9	LEP-14	crispum (Medik.) DC.	unknown	G36	LEP-49	latifolium DC.	Georgia	G63	LEP-104	sativum L. L.	Belarus
G10	LEP-15	crispum (Medik.) DC.	unknown	G37	LEP-50	sativum	Korea	G64	LEP-105	sativum L. L.	Georgia
G11	LEP-16	latifolium DC.	Afghanistan	G38	LEP-51	latifolium DC.	Georgia	G65	LEP-106	sativum L. L.	Azerbaijan
G12	LEP-18	latifolium DC.	unknown	G39	LEP-53	latifolium DC.	Georgia	G66	LEP-107	sativum L. L.	Russia
G13	LEP-19	crispum (Medik.) DC.	unknown	G40	LEP-55	latifolium DC.	Georgia	G67	LEP-108	sativum L. L.	Armenia
G14	LEP-21	latifolium DC.	Georgia	G41	LEP-57	latifolium DC.	Georgia	G68	LEP-110	sativum L. L.	Russia
G15	LEP-22	latifolium DC.	Germany	G42	LEP-59	latifolium DC.	Georgia	G69	LEP-111	sativum L.	Georgia
G16	LEP-23	sativum	Armenia	G43	LEP-60	latifolium DC.	Georgia	G70	LEP-114	sativum L.	unknown
G17	LEP-24	latifolium DC.	Armenia	G44	LEP-61	latifolium DC.	Tajikistan	G71	LEP-115	sativum L. crispum (Medik.) DC.	unknown
G18	LEP-25	sativum	Italy	G45	LEP-62	latifolium DC.	Georgia	G72	LEP-116	sativum	unknown
G19	LEP-26	latifolium DC.	Georgia	G46	LEP-63	latifolium DC.	Georgia	G73	LEP-117	sativum	unknown
G20	LEP-27	latifolium DC.	Georgia	G47	LEP-64	sativum	Georgia	G74	LEP-118	sativum	unknown
G21	LEP-28	latifolium DC.	Georgia	G48	LEP-65	sativum	USSR	G75	LEP-119	latifolium DC.	unknown
G22	LEP-30	latifolium DC.	Georgia	G49	LEP-66	sativum	Germany	G76	LEP-120	latifolium DC. L.	USSR
G23	LEP-31	latifolium DC.	Georgia	G50	LEP-67	sativum	Germany	G77	LEP-126	sativum L. L.	USSR
G24	LEP-32	latifolium DC.	Georgia	G51	LEP-68	sativum	Germany	G78	Birjand	sativum L. L.	Iran
G25	LEP-33	sativum	Libya	G52	LEP-69	sativum	Germany	G79	Kerman	sativum L. L.	Iran
G26	LEP-34	latifolium DC.	Georgia	G53	LEP-70	sativum	Germany	G80	Tabriz	sativum L. L.	Iran
G27	LEP-36	latifolium DC.	Georgia	G54	LEP-72	sativum	Germany	G81	Shiraz	sativum L.	Iran

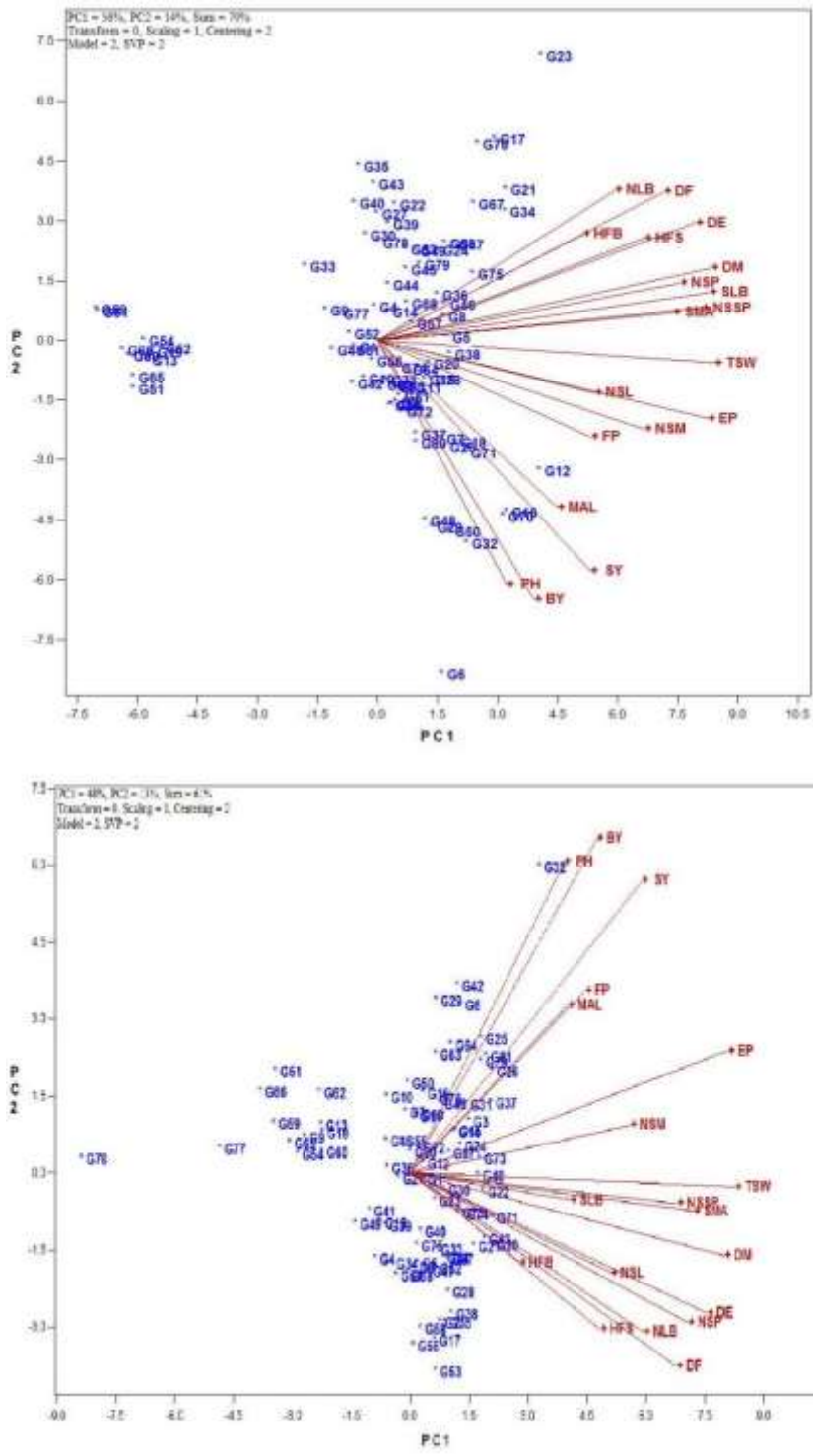


Fig 1: Vector-view of GT biplot showing the relationships among 19 traits of 81 garden cress genotypes: A) for the year 2012 and B) for the year 2013

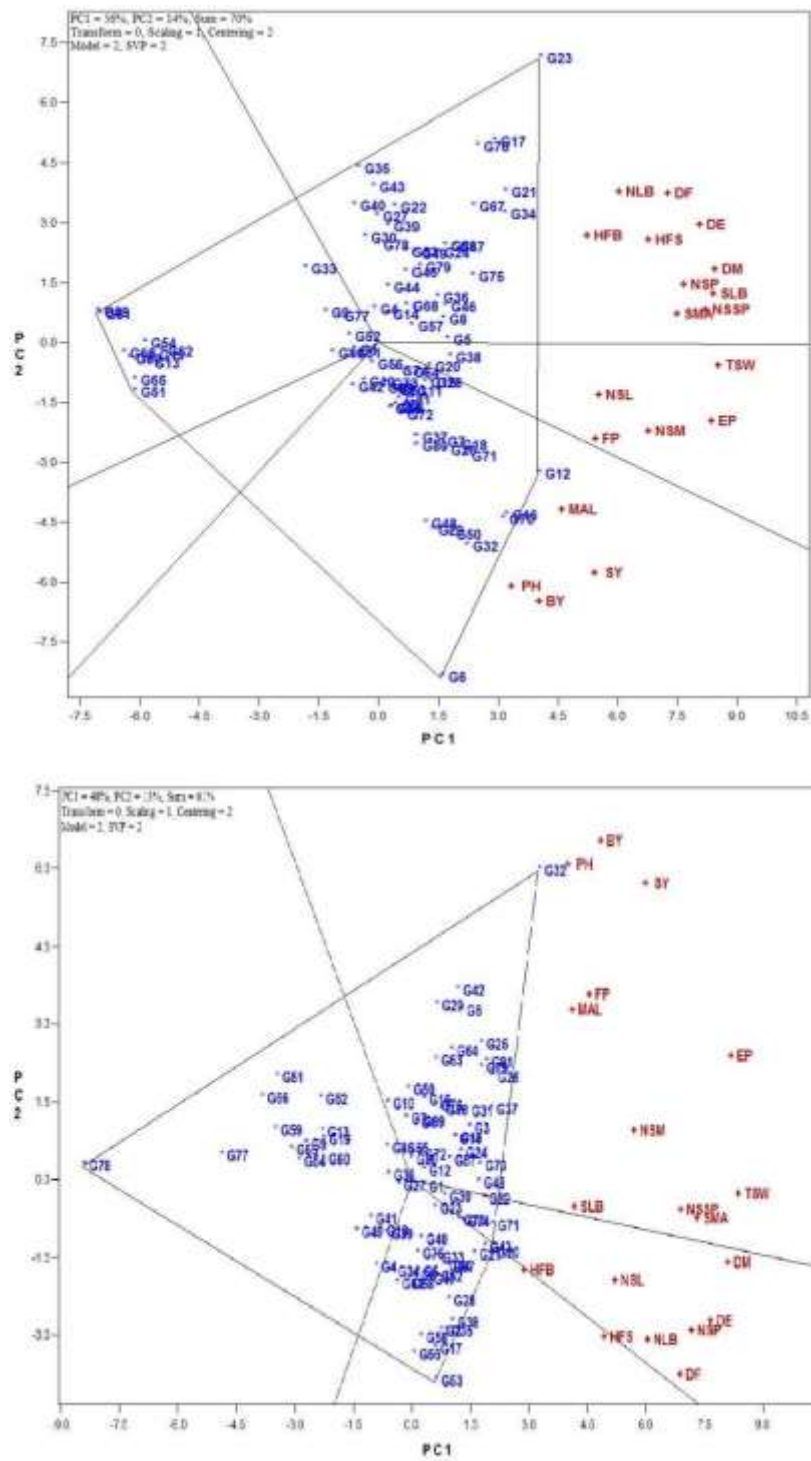


Fig 2: Polygon-view of GT biplot displaying the "which-won-where" pattern of 19 traits and 81 garden cress genotypes: A) for the year 2012 and B) for the year 2013

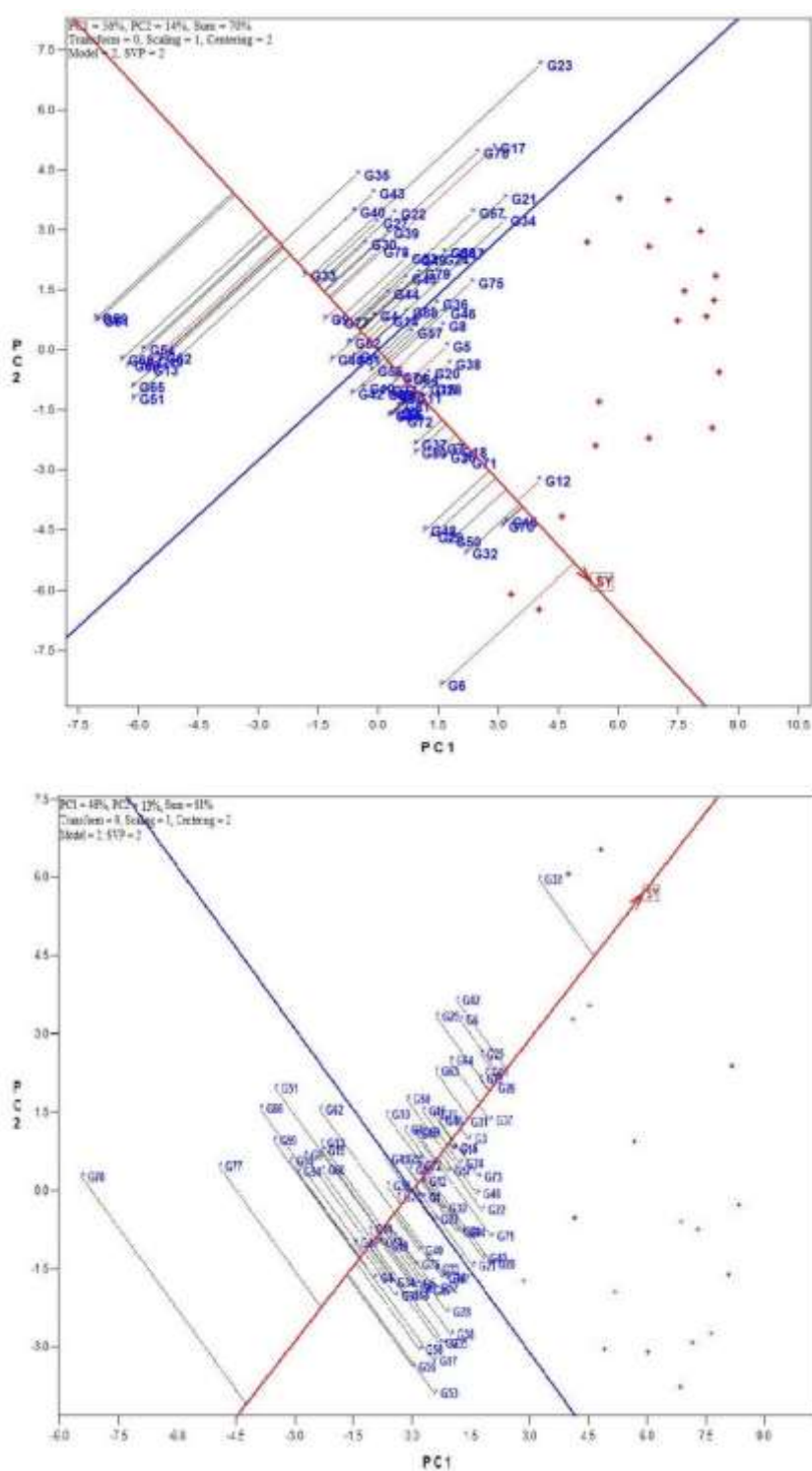


Fig 3: GT biplot illustrating the ranking of 81 garden cress genotypes based on seed yield:
 A) for the year 2012 and B) for the year 2013



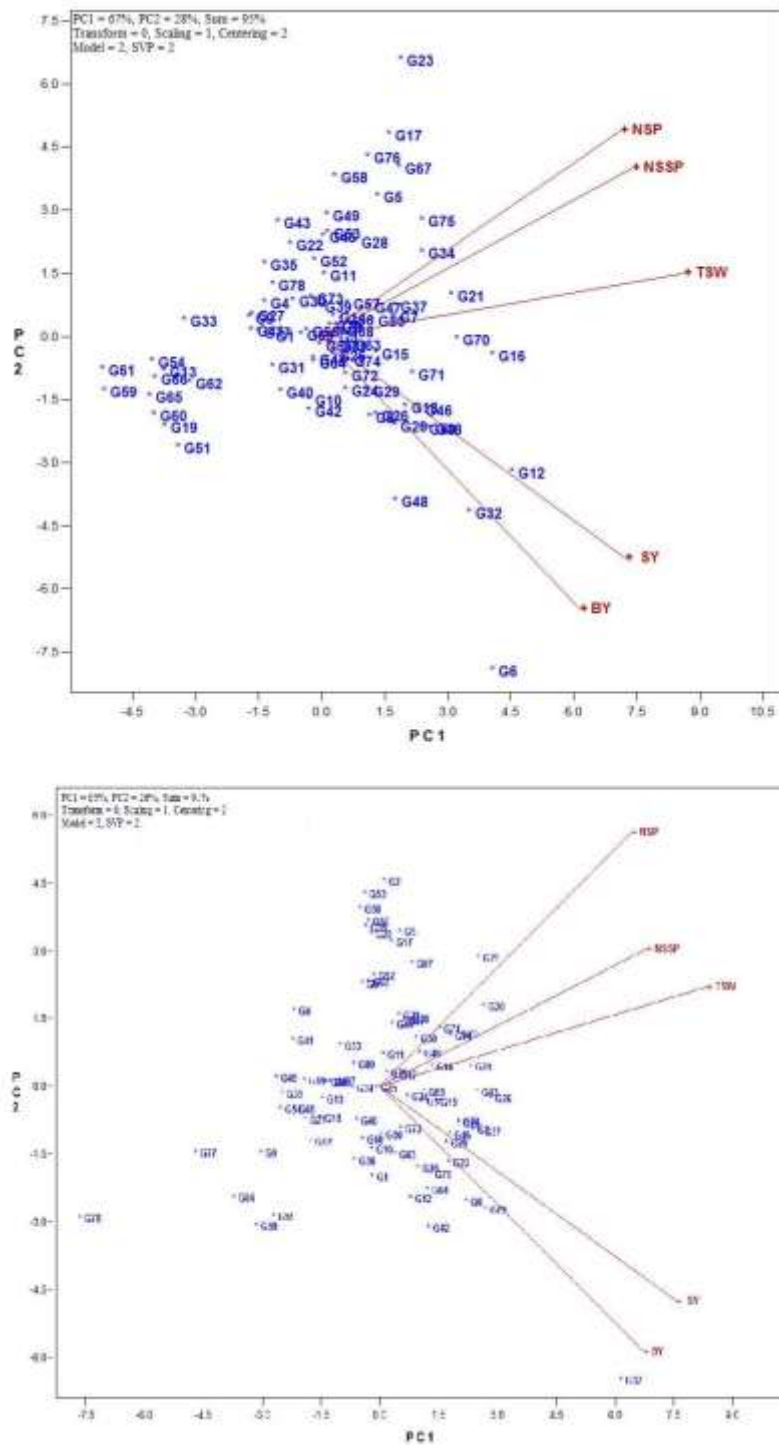


Fig 4: Vector-view of GT biplot representing the relationship among yield components of 81 garden cress genotypes: A) for the year 2012 and B) for the year 2013

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