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Evaluation and Identification of Stable Chickpea Lines for Yield-Contributing Traits from an Association Mapping Panel

Philanim Wungmarong Shimray [1], C. Bharadwaj [1,*], B. S. Patil [1], S. Mukesh Sankar [1], Neeraj Kumar [1], Sneha Priya Pappula Reddy [1], Tripti Singhal [1], Venkatraman Hegde [1], Swarup K. Parida [2], Manish Roorkiwal [3], Rajeev K. Varshney [4] and Preeti Verma [5]

[1] Genetics Division, Indian Agricultural Research Institute (IARI), Pusa Campus, New Delhi 110012, India
[2] National Institute of Plant Genomic Research (NIPGR), Jawaharlal Nehru University Campus, Aruna Asaf Ali Marg, New Delhi 110067, India
[3] Genomic Breeding Lead, Khalifa Center for Genetic Engineering and Biotechnology (KCGEB), UAE University, Al Ain 15551, United Arab Emirates
[4] State Agricultural Biotechnology Centre, Centre for Crop and Food Innovation, Food Futures Institute, Murdoch University, Murdoch 6150, Australia
[5] Department of Plant Breeding and Genetics, Agricultural University, Kota, Rajasthan 313601, India
* Correspondence: drchbharadwaj@gmail.com

Abstract: An association mapping panel consisting of 380 genotypes of chickpea was evaluated for three different years, including 2014–2015, 2015–2016 and 2016–2017, for yield-contributing parameters, including the seed number and seed weight. The AMMI analysis presented mainly concentrated on the seed weight and seed number, which are the two most important yield-contributing traits. The genotypes contributed 93.08% of the total variance, while the interaction effect was comparatively low, with 4.1% for the two traits. AMMI biplot analysis identified IG5986, IG5982, ILC6025 and ICCV14307 as desirable genotypes for the seed weight and IG5893, ILC6891 and IG5856 for the seed number. Identifying stable genotypes would help in strategic planning for yield improvement through component trait breeding.

Keywords: AMMI analysis; chickpea; seed number; seed weight

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1. Introduction

The extent of protein calorie deficiency has rampant effects on the infants and young children of developing and underdeveloped countries. It is also further aggravated by various pathological and physiological abnormalities due to a lack of sufficient protein and calories in the diet [1]. Near about 170 million people, mostly school going children's and lactating mothers in developing countries of Asia and Africa are severely impacted by hidden hunger or malnutrition [2]. As an economic consequence of hidden hunger every year there are losses of up to 11% of gross domestic product (GDP) in African and Asian countries [3].

Legumes are special food crops with high protein value and are, therefore, considered as an indispensable food source to fill in the protein gap globally. Among pulse crops, chickpea is one of the oldest tamed pulse crops about 7450 years back in the regions of Middle East [4]. It is considered as the world's most important legumes, covering a global area of 13.98 Mha and with overall production of 13.7 Mt, it comes second only after dry beans in the world's food legume production [5]. India tops in chickpea production globally, with yearly production of 9.8 Mt, representing 70% of the total world chickpea production from an estimated area of 9.9 Mha [5]. Chickpea offers high nutritional value for humans; it has rich protein value, which ranged from 20-30% in seeds. The dietary fibre, and constitutes approximately 40% carbohydrates and chickpea has up to 3-6% oil content in seed [6]. Further chickpea is also containing significant proportion of essential

micronutrients such as, potassium, calcium, iron, zinc, phosphorus, and manganese making it one of the best composed dry legumes for human health and consumption [7]. Despite global lead in chickpea production as per the current projections of demand and supply, India needs to strengthen its domestic production to curtail increasing imports which is about 186,000 tonnes (USD 74 million) to become self-reliant of chickpea. To achieve that, identification of stable, high yielding and adaptive genotypes in is a necessity.

To achieve this, it is necessary to develop stable genotypes widely adapted to diverse climatic conditions and varying environments. To identify stable genotypes, the knowledge of GEI (genotype–environment interaction) is required, for which AMMI (additive main effects and multiplicative interaction), developed by Gauch, 1988 [8], is the most used method. AMMI analysis provides powerful information on the stability of a genotype in a general environment or in a particular environment, enabling mega-environment delineation. It employs biplots for the comprehension and study of the data. The seed weight and seed number are two important component traits of chickpea that are known to have the highest positive influence on the seed yield per plant [9–11]. The effect of seed size on the yield and yield components is significant for most of the traits, and the seed weight is an important yield determinant [12–16]. A positive correlation between seed size, seed yield and seed weight has also been confirmed, where larger-seeded chickpea has a higher seed yield [17]. Therefore, the component traits, including the seed weight and seed number, were used as the basis for identifying stable genotypes in the current study.

2. Materials and Methods

2.1. Plant Material

The planting material consisted of panel consisted landraces from the WANA region (West Asia and North Africa), training population and released varieties. Total three hundred and eighty genotypes were included in the study. These included 367 *kabuli* and 13 *desi* chickpea types (Table S1). The genotypes used were obtained from ICARDA (genomic population), ICAR-IARI and ICRISAT.

The materials were grown at ICAR, the Indian Agricultural Research Institute Research farm, New Delhi (280°382′ N, 770°802′ E), and laid out in the field in an augmented block design (ABD) with 30 cm spacing between the rows and 10 cm spacing between plants. The trial was undertaken and data were recorded for 3 consecutive years, including 2014–2015, 2015–2016 and 2016–2017 during the cropping season from October to April (Table 1). All the experiments were conducted in three replications with all standard chickpea agronomic practices.

Table 1. Rainfall and temperature data for the chickpea growing seasons for three years.

Cropping Season (October–April)	Rainfall (mm)	Temperature (°C)	
		Minimum	Maximum
2014–15	315.8	6.6	34.8
2015–16	22.0	6.1	38.7
2016–17	127.7	5.3	38.0

2.2. Statistical Analysis

Genstat software (v.18.1, England & Wales) was used to analyse the data. ANOVA was used for seed weight and seed number to determine their statistical significance. Cumulative analysis for the 3 years of data was conducted after testing for the error variance of homogeneity. The AMMI model was used for stability analysis, as suggested by Zobel et al. [18], Gauch [19] and Purchase [20]. AMMI analysis is preferred, as it gives an estimate of the total G × E interaction effect of each genotype and further categorizes it into interaction components due to individual environments.

3. Results

A pooled ANOVA for seed weight and seed number across the years was performed, where different years were taken as random effects and genotypes were considered as fixed effects. The result shows high significance ($p < 0.01$) for seed weight and seed number for genotype (G), environmental (E) and interaction (GEI) effects, thus indicating the roles of all three types of effects, which is merely not random or due to chance (Table 2). The maximum variation was accounted for by genotypic effect due to seed weight and seed number, contributing 93.08% for both, respectively, followed by G × E effects, with 4.1% for the two traits. The minimum variation was accounted for by the environmental influence, with 0.37% for both traits.

Table 2. Pooled ANOVA for seed weight and seed number across the years.

Source	d.f.	Seed Weight		Seed Number	
		MSS	Variance (%)	MSS	Variance (%)
Genotypes	379	606.3 **	93.08	606.3 **	93.08
Environments	2	457.4 **	0.37	457.4 **	0.37
Rep within Env	6	31.7 **		31.7 **	
G × E	758	13.4 **	4.1	13.4 **	4.1
Error	2274	2.6		2.6	
Total	3419	72.2		72.2	

** Highly significant at $p < 0.01$.

3.1. AMMI Analysis

To deduce the appropriateness of the data in AMMI analysis, the genotype environment signal (GEs) was calculated [21]. Subtracting GEn (GE noise) from GEI was done to obtain GEs. For estimating GEN, the error mean sums of squares and degrees of freedom (df) for GE is prerequisite. Thus, in the very first stage included evaluation of GEN by multiplying the error mean sum of squares by the degrees of freedom for GE ($2.6 \times 758 = 1970.8$ for both seed weight and seed number). Further, GEs was computed ($10132 - 1970.8 = 8162.2$ for both seed weight and seed number). When the SS due to GEN is nearly equivalent to the SS due to the GEI obtained in the ANOVA, it is claimed that GEI is buried in the noise and is hence a signal with low quality. However, SS owing to GEN was well below than GEI sum of squares in this investigation. As a result, the interaction virtually had a high signal to noise ratio. This highlighted the study's ability to benefit from the AMMI analysis.

3.2. Ascertaining High-Yielding and Stable Genotypes

To understand the main effects and interactions for seed weight and seed number, an AMMI biplot was constructed (Figures 1 and 2). AMMI biplot is a plot between the mean and the IPCA1 of GEI. The biplot reveals that when the main effects' IPCA scores near to zero, there is little to no interaction between the genotype and the environment. Conversely, when a genotype and environment have the same sign on the IPCA axis, there is positive interaction; if there is a difference, there is negative interaction. In Figure 1, G369 was identified as the most stable genotype for seed weight, with the IPC1 score nearing zero (−0.009) and the mean seed weight being 37.33 g/100 seeds.

For the seed number per plant, the genotype with the highest mean seed number was G182 (57.67), but it was less stable (IPCA1 score of −0.78). The most stable genotype was G57, with an IPCA1 score close to zero (−0.03) and mean seed number of 38.44. Further, the most unstable genotype was identified to be G301, with a mean seed number of 27.11. Genotypes G80 and G140 showed good stability (scores of −0.28 and −0.37, respectively) with a high seed number (50.44 and 53.56, respectively).

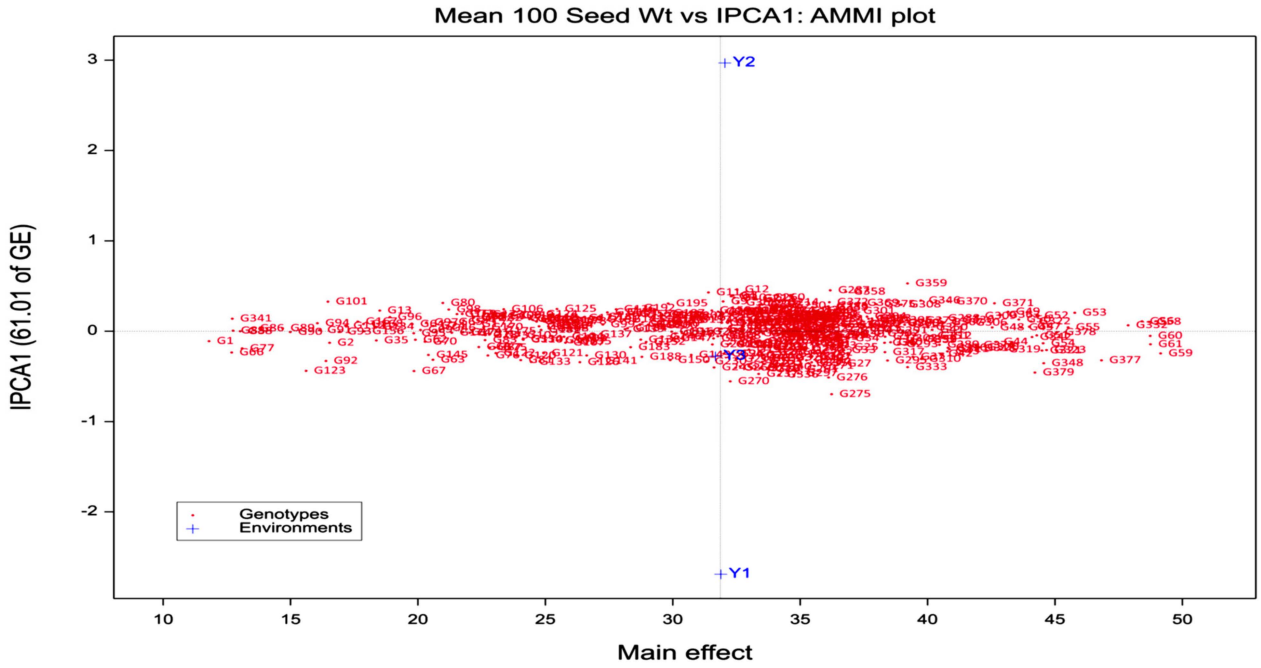

Figure 1. AMMI1 biplot for seed weight.

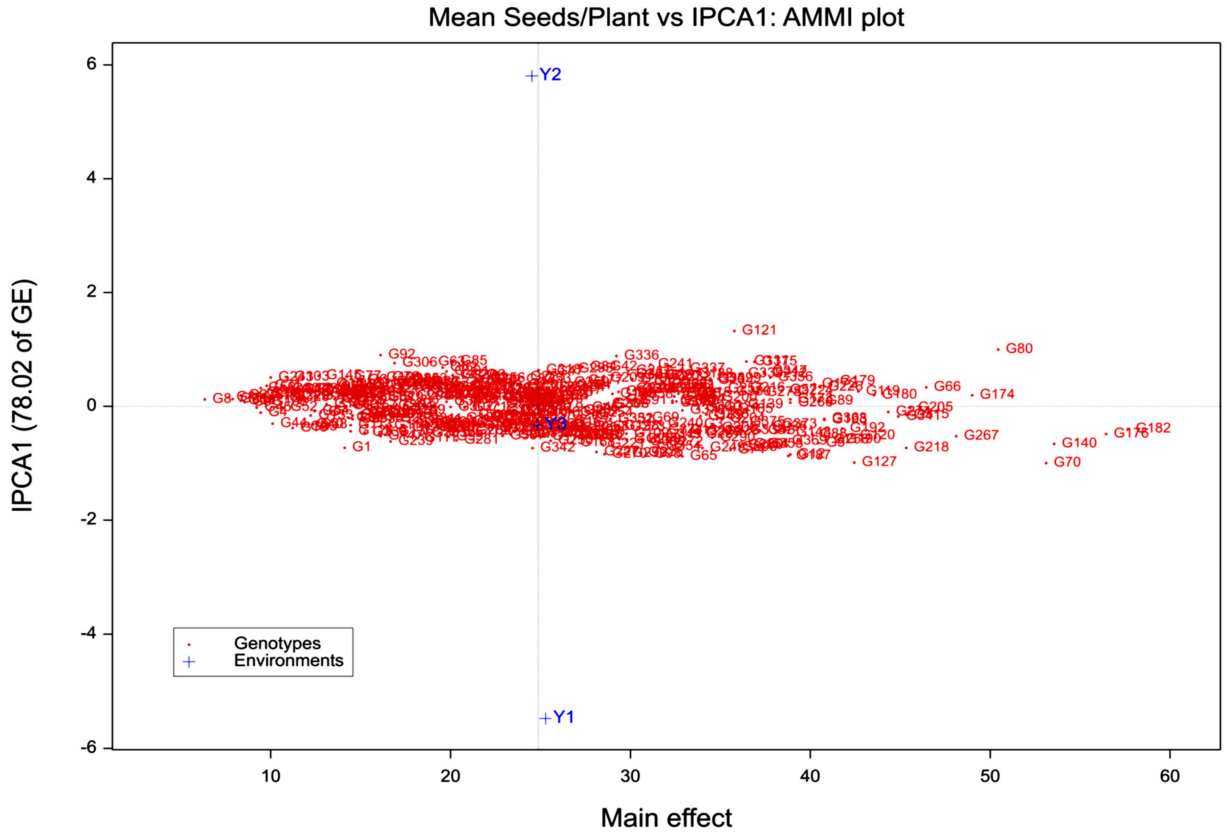

Figure 2. AMMI1 biplot for seed number. G59 had the highest mean seed weight (49.1 g) with good stability (IPC1 score of −0.28). G60 and G61 had high mean seed weights (48.7 and 48.7, respectively) and IPC1 scores nearing zero (−0.09 and −0.25, respectively). The most unstable genotype identified was G182 (IPC1 score of −0.78), with a mean seed weight of 24.8 g.

3.3. AMM2 Biplot

This biplot, which contrasts IPCA1 and IPCA2, illustrates how strongly each genotype interacts with the environment. In the biplot, for seed weight, IPC1 accounted for 61.01% of the interaction and IPC2 accounted for 38.99%. Further, for seed number, the first component, IPC1 explained 78.02% of the genotype and environment interaction and IPC2 described 21.98%. For both traits, the first two interaction components explained 100% of the G × E variation, leaving no residue or noise (Table 3).

Table 3. ANOVA for AMMI2 model for seed weight and seed number.

Source	d.f.	Seed Weight			Seed Number		
		MSS	% GE Explained	% Cumulative	MSS	% GE explained	% Cumulative
Treatments	1139	211.5 **			211.5 **		
Genotypes	379	606.3 **			606.3 **		
Environments	2	457.4 **			457.4 **		
G X E	758	13.4 **			13.4 **		
IPCA1	380	24.4 **	61.01	61.01	24.4 **	78.02	78.02
IPCA2	378	2.3 **	38.99	100	2.3 **	21.98	100
Residual	0	0			0		
Error	2274	2.6			2.6		
Total	3419	72.2			72.2		

** Highly significant at $p < 0.01$.

The scattered genotypes near the origin indicate minimum interaction of these genotypes with the environment. The distances from the origin indicate the amount of interaction that occurred between genotypes and environments or vice versa [22]. From the biplot, G378, G34, G22, G85, G289, G312, and G32 were scattered close to the origin indicating minimal interactions with the environment for seed weight (Figures 3 and 4). Genotypes that were scattered far away from the origin including G275, G201, and G2, showed prominent G × E interactions; thus, there were less stable. For seed number, higher sensitivity to the environment was shown by genotypes G70 and G267.

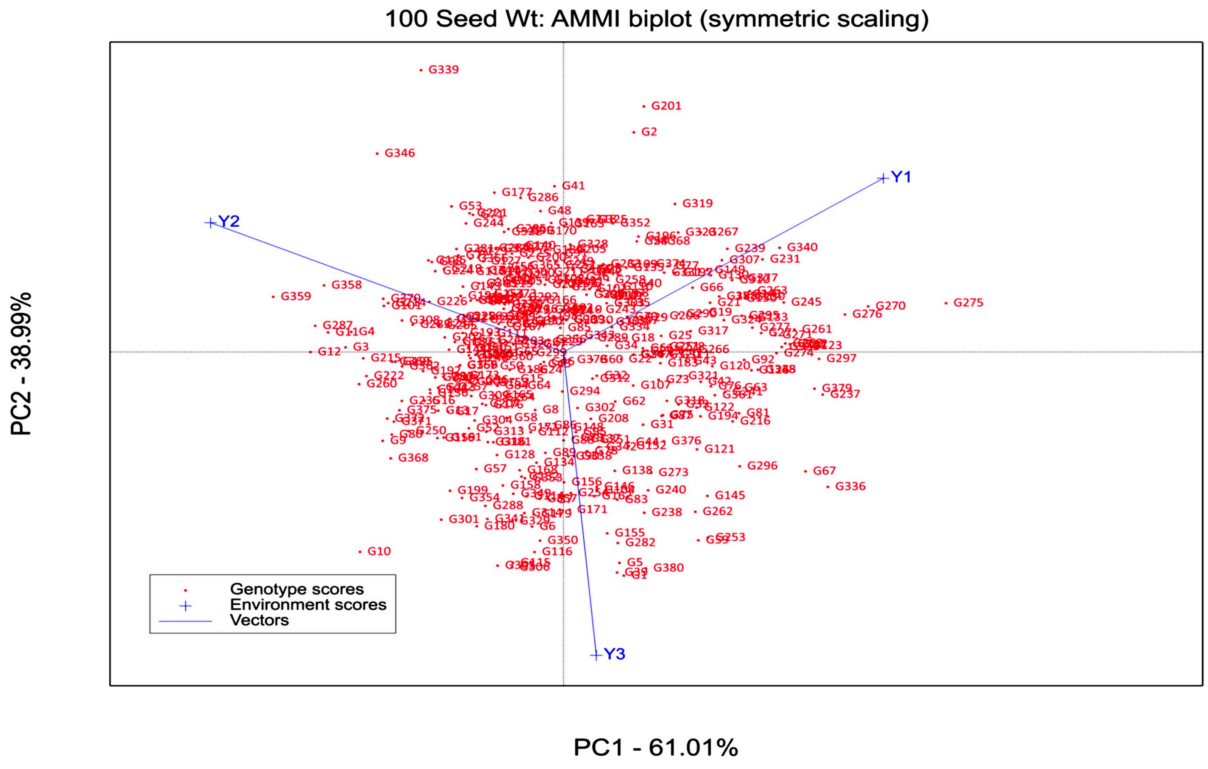

Figure 3. Seed weight—AMMI2 biplot of IPCA1 vs. IPCA2.

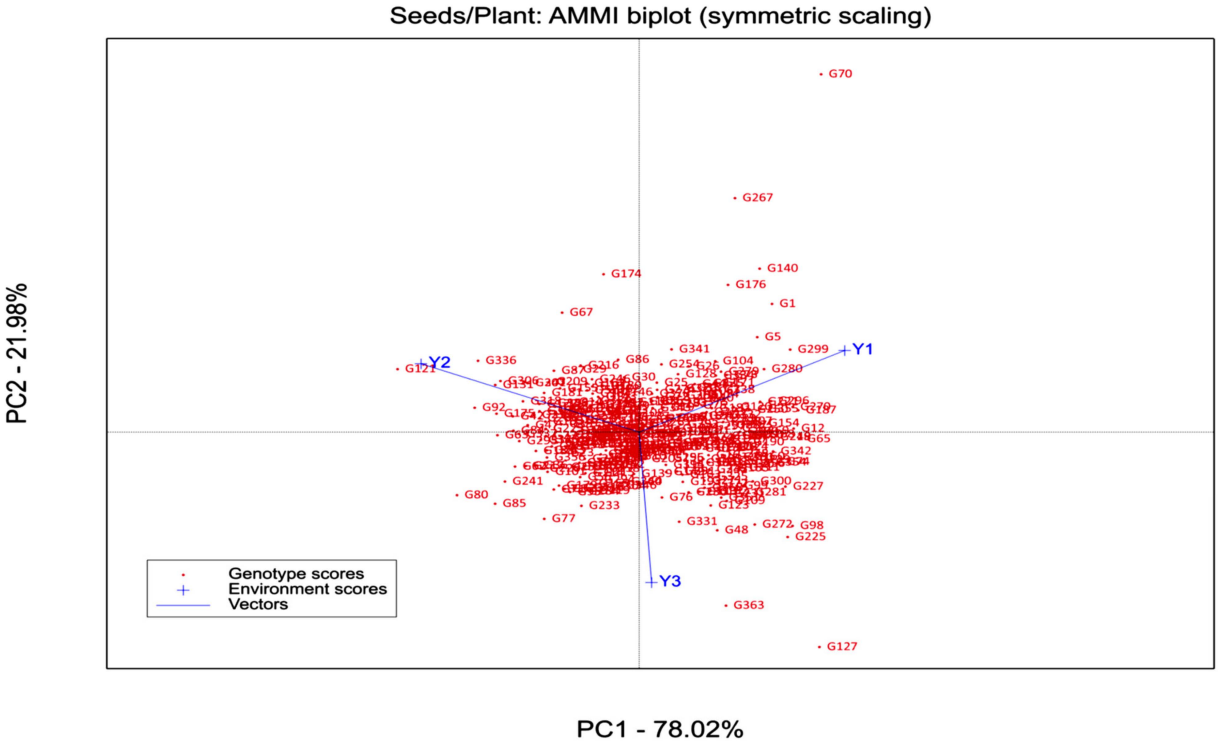

Figure 4. Seed number—AMMI2 biplot of IPCA1 vs. IPCA2.

4. Discussion

The AMMI analysis of variance showed that the genotype, environment and interaction effects were significant ($p < 0.01$), indicating differences in the genotype's behaviour in the environments. This justifies understanding genotype behaviour in order to justify the magnitude and extent of their interactions with the environments [19]. The phenotypic stability in this study was estimated by the significance of the GE interaction [23,24]. Selection for yield stability across environments defined by location year combinations would aid in dealing with genotype–year or genotype–location–year interactions [25]. Many earlier reports of AMMI analysis studies in rice utilized these models to get gain a deeper insight into the environmental factors in conjugation with G × E interactions. They also provided the possibility of predicting the performance of different varieties in environments where they have not been tested, and of improving the precision of the estimation of genotype and environment effects [26–28]. Studies in wheat by Nachit et al. [29], also concluded that, multilocation testing results in significant genotype–environment (GE) interactions, lowering true yield estimation accuracy. Thus, AMMI analysis effectively eliminated the random variation and truly measured the genotypic estimates, which were more precise than unadjusted means and crucial for estimating the true genotypic value. Similar results were also reported for evaluating genotypes and analysing G × E for plant yield in wheat by Rad et al. [30].

Similarly, for the identification of micronutrient-enriched and highly stable pearl millet genotypes along with higher yields both AMMI and GGE biplot analyses were used, demonstrating the possibility of improving the micronutrient content without compromising the grain yield [31,32]. In chickpea, also for analyzsing the most stable genotype with wide adaptability across diverse environments, AMMI analysis were used to understand the GE interaction pattern. The biplot of the first principal component and mean grain yields for genotypes and environments revealed that high-yielding genotypes were not cultivars with the sustained yield [33]. For seed yield, it was found to be contributed by higher G × E variations and thus differential genotypic performance is observed over different environments [34]. Different methods have been developed to estimate significant genotypes by environment interaction (GEI) in chickpea [35].

All of these researchers discovered a significant G × E interactions for grain yield and emphasized the value of AMMI analysis in identifying and selecting promising stable genotypes for specific locations or environmental conditions. ANOVA revealed the maximum variation explained by the genotypic effect similar to the studies by Akter et al. [36] in rice and Anuradha et al. [32] in pearl millet. A contradictory observation was reported by Saboghpour et al. [33], Balapure et al. [34] and Kanouni et al. [35] where the largest contribution to the total variation was from environmental effects and the genotype had little effect. The AMMI model is useful because it uses overall fitting, imposes no restrictions on the multiplicative terms, and produces the least-square fit [37]. Gauch and Zobel [38] emphasized the usefulness of AMMI1 with IPCA1 and AM-MI2 with IPCA1 and IPCA2 biplots, as well as the graphical representation of axes, either as IPCA1 or IPCA2 against main effects or IPCA1 against IPCA2. The first two IPCs, IPCA1 and IPCA2 could explain 100% of the interaction effect for the seed weight and seed number per plant leaving no residue. This observation supports Gauch and Zobel's [38] findings that the most accurate model for AMMI can be predicted using the first two IPCAs. G369 and G57 were identified as the most stable genotypes for the seed weight and seed number per plant, as deduced from their IPC scores in the biplot. A genotype is best suited to a given environment when it presents highly positive interactions with the specific environment making it invariably more suited to exploiting the ecological and management conditions of the environment. The study of G × E interaction discerns the stability of a genotype in different environments and the above study clearly showed the convenience of the AMMI model in deciphering the most stable and most unstable genotypes for different environments. It will further aid in developing environment-wise adaptable genotypes, depending on the extent of the genotypes' interactions with the environment. Less interactive genotypes for general adaptation and more interacting genotypes for specific environments can be identified from this study.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12123115/s1, Table S1: List of the genotypes used in the association panel (K—*kabuli*; D—*desi*).

Author Contributions: P.W.S., B.S.P. and S.P.P.R. conducted the phenotyping and genotyping work; S.M.S., N.K., T.S. and S.K.P. conducted the statistical analysis; C.B., R.K.V. and V.H. devised the work and study plan; M.R. and P.V. conducted the interpretations; P.W.S. and C.B. conducted the manuscript preparation; and all authors contributed to manuscript development and review. All authors have read and agreed to the published version of the manuscript.

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