DEPARTMENT OF AGRICULTURAL, FOOD, AND ENVIRONMENTAL SCIENCES

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CHARACTERIZATION OF A DIVERSE CHICKPEA (*CICER ARIETINUM* L.) GERMPLASM PANEL FOR CRUDE PROTEIN CONTENT

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ABSTRACT

Chickpea (*Cicer arietinum L.*) is a source of protein, playing a significant role in sustainable agriculture and food security. However, understanding how different chickpea genotypes respond to diverse environmental conditions is crucial for breeding programs that enhance nutritional stability, particularly for protein content. This study evaluated 202 chickpea genotypes from the EMCAP panel grown across two environments in 2019 and 2021 to assess crude protein content variability, genotype-by-environment interaction (GEI), and broad-sense heritability (H^2). Using random effect models and variance component analysis, we estimated the contribution of genotype, environment, and GEI to total phenotypic variance.

Results revealed significant genotypic and environmental effects on protein content, with genotype and environment explaining 10.60% and 9.47% of the total variance, respectively. The GEI contributed 23.17%, highlighting differential genotype responses across environments. Residual variance was the largest component at 56.66%, suggesting substantial environmental variation within each trial. Broad-sense heritability for protein content was moderate, indicating that while genetic factors contribute to protein levels, environmental effects are equally impactful. Additionally, a stability analysis identified genotypes with consistent protein levels across environments, which are promising candidates for breeding programs focused on enhancing protein stability in chickpeas.

This study underscores the importance of accounting for environmental variability in chickpea breeding and offers insights into selecting genotypes with high protein content and stability across different environments. These findings are crucial for developing resilient chickpea varieties adapted to diverse growing conditions, supporting global food security goals.

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1 INTRODUCTION

1.1 LEGUME CHARACTERISTICS

Legumes, part of the Fabaceae or Leguminosae family, form the third-largest group of flowering plants. They are surpassed only by the Orchidaceae (orchids) and Asteraceae (daisies). According to Lewis et al. (2005), this family encompasses 727 genera and 19,325 species. Legumes grow in various ecological conditions, ranging in form from small herbs to towering forest trees. Taxonomists have categorized the Fabaceae family into three main subfamilies, primarily distinguished by flower characteristics. These subfamilies vary in size and distribution.

Traditionally, the classification includes the Papilionoideae with 476 genera and around 14,000 species, the Caesalpinioideae with 162 genera and approximately 3,000 species, and the Mimosoideae with seventy-seven genera and about 3,000 species (Doyle & Luckow, 2003). The Caesalpinioideae subfamily is predominantly composed of tropical and subtropical trees and shrubs. In contrast, the Mimosoideae subfamily consists largely of trees and shrubs, including the timber-producing *Acacia* genus (International Legume Database and Information Service, 2007).

The Leguminosae family ranks second only to the Gramineae in terms of importance in agriculture. Grain legumes contribute 33% to the protein requirements of the human diet (Graham & Vance, 2003). While cereals are an excellent source of carbohydrates for energy, legumes serve as the primary source of dietary protein for a massive portion of the global population. Soybeans, groundnuts, and other grain legumes provide heart-healthy and antioxidant nutrients. Regular consumption of legumes is associated with a reduced risk of various health conditions, including cardiovascular disease, stroke, Parkinson's, Alzheimer's, Huntington's diseases, liver disorders, and cancer (Singh, 2007).

Legumes also play a significant role in global trade. Grain legumes, which are rich in proteins, combined with cereals, which are high in carbohydrates, form an ideal combination for a balanced diet. Green legumes can be consumed as vegetables by humans, used as animal fodder, and contribute to soil improvement through nitrogen fixation or as green manure (Hasanuzzaman et al., 2019). They are used not only for human consumption and animal feed but also in various industries, such as pharmaceuticals, soap, paints, resins, coatings, linoleum, cosmetics, lubricants, chemicals, plastic coatings, and ethanol production. Increased protein intake is essential to overcome protein-energy malnutrition (PEM), which affects one in

four children globally. Approximately 70% of children impacted by PEM live in Asia, 26% in Africa, and 4% in Latin America and the Caribbean (World Hunger Education Service, 2006). Increasing the intake of plant-based proteins could help address PEM, particularly among vegetarian populations (Singh, 2007).

Recent FAOSTAT data highlights global agricultural trends, showing continued growth in crop production, with cereals, oilseeds, and pulses playing major roles. Total cereal production reached 2.8 billion tons, dominated by maize, wheat, and rice, cultivated across 720 million hectares globally. India remained the largest producer of legumes in 2022, contributing approximately 26 million tons to a global total of 96 million tons, followed by Brazil and China with 4.5 million tons each, the United States at 3.7 million tons, and Canada at 3.2 million tons (FAOSTAT, 2022). Soybeans continue to dominate legume cultivation, achieving a record global yield of 360 million tons, primarily produced by Brazil, the United States, and Argentina. Other legumes, including beans, peas, and chickpeas, maintain significant production volumes. Brazil, India, and China are leading in bean cultivation, contributing around 30 million tons from 25 million hectares worldwide. Pea production, with a stable yield of approximately 210 million tons, is distributed widely, with notable contributions from Canada, the USA, and the European Union. Canada and India lead lentil production, accounting for a combined 6.8 million tons globally. Chickpeas, largely produced in India, reached an estimated global output of 12 million tons, underscoring their importance in global diets and plant-based protein demand (FAOSTAT, 2022). (Table 1.1)

Table 1.1 Crop production and area harvest. Source: FAOSTAT 2022

Crop	Production (Million Tons)	Harvested Area (Million Hectares)	Top Producers
Cereals	2800	720	USA, China, India
Oilseeds (Soybeans)	360	120	Brazil, USA, Argentina
Pulses (Chickpeas)	12	14	India, Australia, Turkey
Pulses (Beans)	30	25	Brazil, India, China
Pulses (Lentils)	6.8	6	Canada, India, Australia
Pulses (Peas)	210	15	Canada, EU, USA

Many legume crops can fulfill multiple roles depending on their maturity at harvest (Figure 1.1). Grain legumes are grown for their seeds, which are consumed by humans and animals or processed to extract oils for industrial applications. The term "pulse" refers to legume species that are specifically grown for their dried seeds. Pulses like lentils, beans, peas, and chickpeas are essential to the global food supply, playing a

key role in meeting nutritional needs in a cost-effective manner. This is particularly important in developing and underdeveloped nations where access to animal protein is limited or too expensive (Aguilera et al., 2009).

Food legumes are a highly nutritious source of protein and bioactive compounds that offer significant health benefits. The World Health Organization (WHO) recommends legumes for managing chronic non-communicable diseases like diabetes and heart disease (Hosseinpour-Niazi et al., 2015). Their low-fat content and sterols have been shown to effectively lower LDL cholesterol and reduce blood pressure (Hosseinpour-Niazi et al., 2015). Grain legumes help smallholder farmers by increasing crop yields, providing an affordable source of protein that makes up 15% of protein consumption, and serving as a cash crop-ranking as the third-largest export crop after coffee and sesame (Getachew, 2019). Additionally, legumes contribute to improving soil fertility through biological nitrogen fixation (BNF), provide livestock feed, control soil erosion, offer a source of fuel, and deliver various other environmental and economic benefits (Muoni et al., 2019).

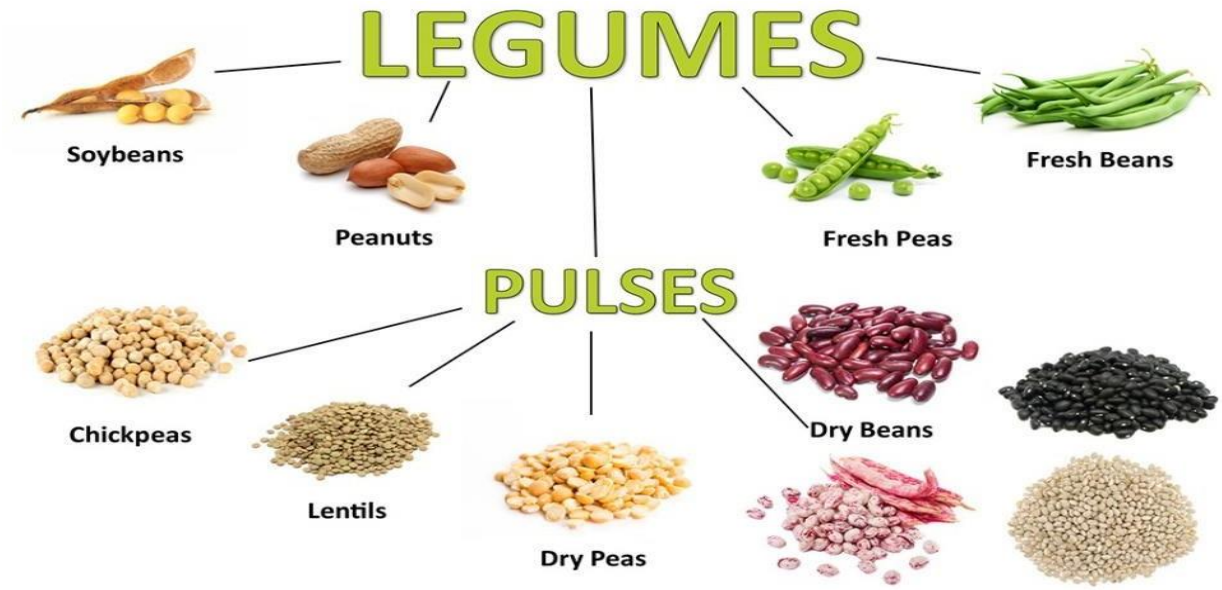

Figure 1.1 Different types of legumes and pulses.

1.2 CENTER OF ORIGIN AND DOMESTICATION OF CHICKPEA

Chickpea (*Cicer arietinum* L.) is the only cultivated species within the *Cicer* genus and is a self-pollinating diploid crop (2n = 2x = 16). Fossil and archaeological evidences suggest that chickpea originated in the Fertile Crescent, specifically in southeastern Turkey and nearby Syria (van der Maesen, 1987). This region, home to the proposed wild ancestor *Cicer reticulatum* (Ladizinsky and Adler, 1976b; Ahmad et al., 1992; Iruela et al., 2002), has yielded archaeological findings of chickpea seeds dating back to around 5,450 BC at Hacilar near Burdur in Turkey (Helbaek, 1970). Further support for its origin comes from the fact that the wild ancestor, *C. reticulatum*, is found only in parts of southeastern Turkey and neighboring Syria, suggesting that domestication could be occurred in this region around 11,000 years ago (Hirst, 2019).

The domestication of chickpea is marked by archaeological evidence from numerous early sites, including Pre-Pottery Neolithic settlements such as Tell el-Kerkh (ca. 8,000 BC) and Dja'de (11,000–10,300 cal BP) in Syria, Cayönü (7250–6750 BC), Hacilar (ca. 6700 BC), and Akarçay Tepe (7280–8700 BP) in Turkey, and Jericho (8350 BC to 7370 BC) in the West Bank (Hirst, 2019). These findings provide compelling evidence of chickpea cultivation's ancient history and its spread through early human agricultural practices.

Chickpeas were an important crop for the Pre-Pottery Neolithic culture, one of the earliest farming societies (Hirst, 2019). After domestication, chickpeas spread westward across the Mediterranean and eastward to South Asia and Ethiopia (van der Maesen, 1987). Domesticated chickpeas have also been introduced to newer regions such as Mexico, Argentina, Peru, Chile, Australia, and the USA (Duke, 1981). Emerging chickpea producers today include Ethiopia, Iraq, Israel, Jordan, Syria, Canada, Morocco, Malawi, and Tanzania. The *Cicer* genus itself comprises 34 perennial species and nine annual species (Rasool et al., 2015).

Domestication led to several beneficial traits in chickpea. While wild varieties ripen only in winter, domesticated chickpea can be planted in spring for summer harvests, reducing the risk of crop failure. Despite its adaptability, chickpea still grows best in cooler winter months, though they remain vulnerable to *Ascochyta* blight, a disease that can devastate crops. Additionally, domesticated chickpea contains nearly twice the tryptophan found in wild varieties. Tryptophan is an amino acid linked to higher serotonin levels, which is associated with improved birth rates and growth in both humans and animals (Hirst, 2019).

Chickpea seeds are classified into two broad categories or market types, *microsperma* and *macrosperma*, based on seed size, like the classification of lentils (Cubero, 1987). Indeed, chickpeas are divided into kabuli and desi types (Figure 1.2). Kabuli types are primarily grown in Mediterranean countries, West Asia, North Africa, Australia, and North America, while desi types are common in South Asia, Iran, Ethiopia, Mexico, and Australia. Despite these morphological differences, all cultivated chickpeas share the same genome (Ahmad et al., 2005).

Figure 1.2 examples of desi and kabuli chickpea seeds.

1.3 TAXONOMY OF CHICKPEA

The cultivated chickpea species are taxonomically classified within the genus *Cicer*, which is part of the Fabaceae family, and the monogeneric tribe Cicereae Alef. (Kupicha, 1981). The genus *Cicer* currently includes 43 species (Table 1.2), divided into four sections—*Monocicer*, *Chamaecicer*, *Polycicer*, and *Acanthocicer*—based on their morphology, life cycle, and geographic distribution (van der Maesen, 1987). Eight of these species, which share the annual growth habit with chickpeas, are particularly important for breeding. Of the nine annual *Cicer* species, eight fall within the *Monocicer* section, while one, *C. chorassanicum*, belongs to the *Chamaecicer* section (Kazan and Muehlbauer, 1991; Muehlbauer, Kaiser, and Simon, 1994). The remaining thirty-three species are perennial, with the life cycle of *C. laetum* Rass. & Sharip being unspecified (van der Maesen, 1987).

Annual Species	**Perennial Species**
C. bijugum K.H. Rech.	*C. anatolicum* Alef.
C. chorassanicum (Bge.) M. Pop.	*C. atlanticum* Coss. ex-Maire
C. cuneatum Hochst ex Rich.	*C. balcaricum* Galushko
C. echinospermum P.H. Davis	*C. baldshuanicum* (M. Pop.) Lincz.
C. judaicum Boiss.	*C. canariense* Santos Guerra & Lewis
C. pinnatifidum Jaub. & Sp.	*C. fedtschenkoi* Lincz.
C. reticulatum Ladiz.	*C. flexuosum* Lipsky
C. yamashitae Kitamura	*C. floribundum* Fenzl
	C. grande (M. Pop.) Korotk.
	C. heterophyllum Contand. et al.
	C. incanum Korotk.
	C. incisum (Willd.) K. Maly
	C. isauricum P.H. Davis
	C. kermanense Bornm.
	C. korshinskyi Lincz.
	C. macracanthum M. Pop.
	C. microphyllum Benth.
	C. mogoltavicum (M. Pop.) Koroleva
	C. montbretii Jaub. & Sp.
	C. multijugum van der Maesen
	C. nuristanicum Kitamura
	C. oxyodon Boiss. & Hoh.
	C. paucijugum (M. Pop.) Nevski
	C. pungens Boiss.
	C. rassoulianum Lincz.
	C. rechingeri Podlech
	C. songaricum Steph. ex DC.
	C. spiroceras Jaub. & Sp.
	C. stapfianum K.H. Rech.
	C. subaphyllum Boiss.
	C. tragacanthoides Jaub. & Sp.

Table 1.2 List of all known species in the Cicer genus. Source: van der Maesen (1987)

1.4 CHICKPEA: AREA OF PRODUCTION AND YIELD (FAOSTAT)

The chart (Figure 1.3) for 2022 illustrates the global distribution of production share of Chickpeas, with Asia dominating at 83.9%, highlighting its role as the primary producer. Oceania follows with 5.9%, while Africa contributes 4.3%. The Americas account for 3.2%, and Europe has the smallest share at 2.7%. The data, sourced from FAOSTAT (November 15, 2024), emphasizes Asia's overwhelming contribution compared to the modest outputs of other regions. While the latest data on chickpea production (Figure 1.4) highlights India as the world's leading producer, contributing around 13.5 million metric tons, despite a slight decline from previous years due to adverse weather. Australia also stands out with significant growth, producing approximately 1.3–2 million metric tons, benefiting from favorable weather and increased planting areas. Other notable producers include Türkiye, with 580,000 tons, and Ethiopia, which produces roughly 493,000 tons annually. Global production trends show rising interest in chickpea cultivation due to its resilience and importance as a staple food. Australia's chickpea area increased by over 80%, reflecting high margins and favorable conditions, while regions like Tanzania and Russia contribute smaller but stable amounts to global supply.

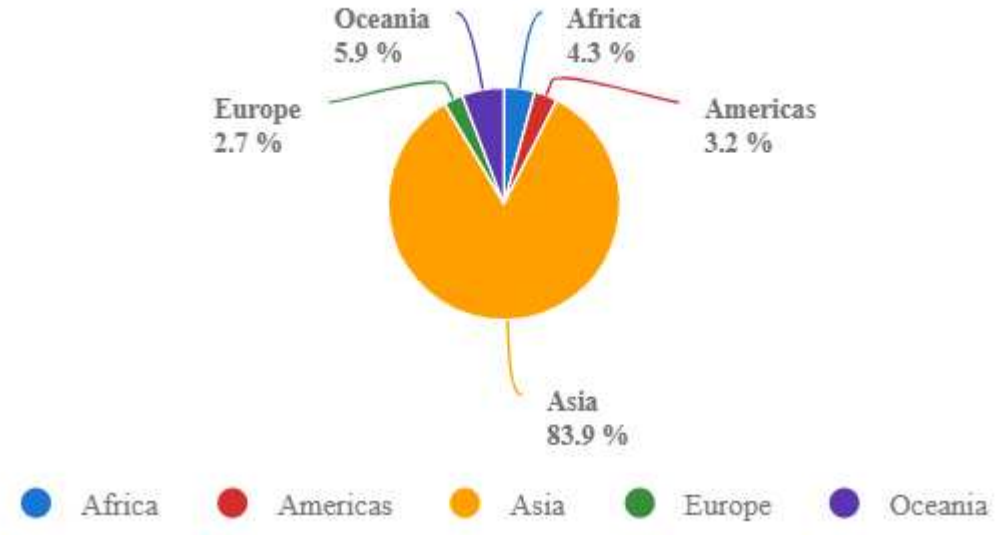

Figure 1.3 A pie chart showing the production of chickpea among the five growing regions in the world (FAOSTAT, 2022)

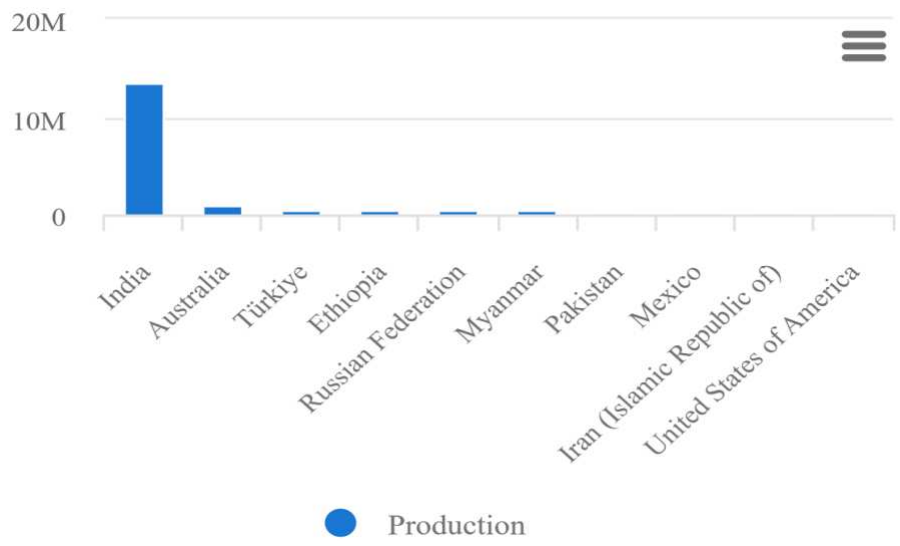

Figure 1.4 A histogram showing the top Ten (10) Chickpea producing countries in the world (Source of data: FAOSTAT, 2022)

The bar chart (Figure 1.5) displays the worldwide production and harvested area of dry chickpeas in 2022. The blue bar represents the area harvested (approximately 14.5 million hectares), and the red bar shows the total production (approximately 18.1 million tons). The chart visually compares the land used for chickpea cultivation to the resulting dry chickpea yield.

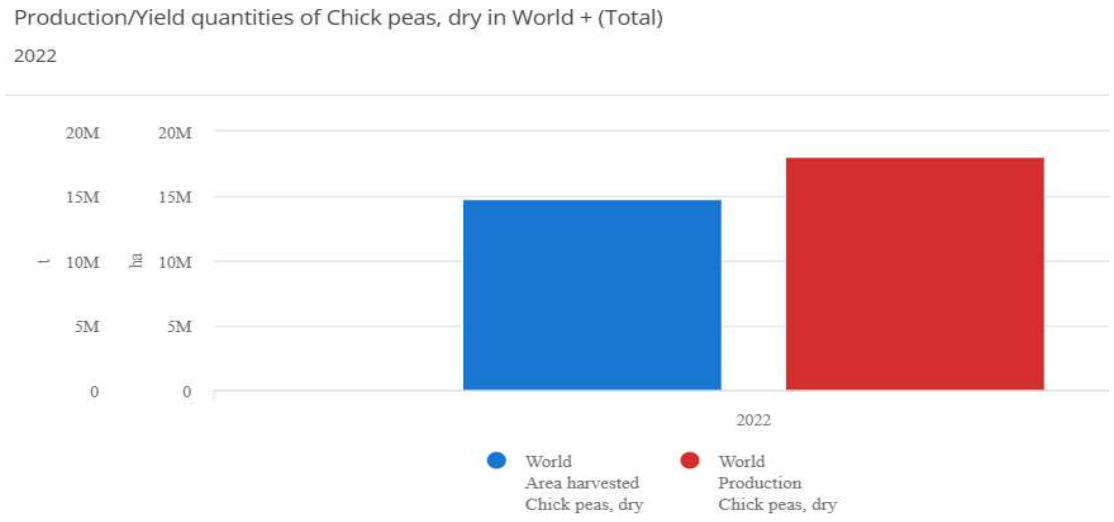

Figure 1.5 The worldwide production and harvested area of dry chickpeas in 2022 (Source of data: FAOSTAT, 2022)

1.5 NUTRITIONAL AND MINERAL COMPOSITION OF CHICKPEAS

1.5.1 General overview

Chickpea seeds are rich in water-soluble vitamins such as riboflavin, pantothenic acid, and pyridoxine, with levels comparable to or higher than those in other pulses. They also have β-carotene, the most important carotenoid that is converted to vitamin A (Jukanti et al., 2012). On a dry weight basis, chickpea seeds have a higher concentration of β-carotene than golden rice endosperm or red wheat. Chickpea offers significant health benefits, reducing the risk of chronic diseases, which makes them a functional food beyond their role as a protein and fiber source (Rasool et al., 2015).

Among the most notable oligosaccharides in chickpea, there are raffinose, stachyose, ciceritol, and verbascose, with ciceritol being the most abundant, hence the name "cicer" for the chickpea plant (Guillon & Champ, 2002). Starch is the primary polysaccharide in chickpea seeds. Chickpea also provides essential minerals like calcium, magnesium, potassium, phosphorus, sulfur, chlorine, boron, iron, manganese, zinc, copper, nickel, and molybdenum, all crucial for human growth and development. Furthermore, they are a reliable source of vitamins, including A, B-complex, C, K, and E (USDA, 2020).

Chickpeas have numerous bioactive compounds, important vitamins, and minerals. However, due to certain antinutritional factors, they require processing before consumption. Traditional methods like soaking, boiling, cooking, germination, roasting, fermentation, and dehulling all influence the bioavailability of their nutrients (Yegrem, 2021).

According to the USDA (2020), cooked chickpea seeds provide approximately 164 calories per one hundred grams, making them a low-calorie yet nutrient-dense food choice (Table 1.5). They are rich in protein, having about nine grams per serving, which contributes to muscle health and satiety. Additionally, chickpea seeds are an excellent source of dietary fiber, with approximately 7.6 grams per one hundred grams, aiding in digestive health and potentially lowering the risk of chronic diseases such as diabetes and cardiovascular disorders. The carbohydrate content is around twenty-seven grams, primarily composed of complex carbohydrates that provide sustained energy. Chickpeas also hold essential micronutrients, including iron, magnesium, and folate, which are crucial for various bodily functions. Their

low glycemic index further makes them suitable for individuals managing blood sugar levels. Overall, the nutritional profile of chickpea supports their inclusion in a balanced diet, promoting health and wellness.

Chickpeas
Nutrition Facts

Serving size 100 g		DV
Calories	**164 kcal**	**8%**
Total Carbohydrate	**27.4 g**	**9%**
Dietary Fiber	7.6 g	30%
Sugars	4.8 g	
Total Fat	**2.6 g**	**4%**
Saturated Fat	0.3 g	1%
Protein	**8.9 g**	**18%**
Vitamin A	27 IU	1%
Vitamin C	1.3 mg	2%
Thiamin	0.1 mg	8%
Riboflavin	0.1 mg	4%
Vitamin B6	0.1 mg	7%
Folate	172 µg	43%
Pantothenic Acid	0.3 mg	3%
Choline	42.8 mg	
Calcium	49 mg	5%
Iron	2.9 mg	16%
Magnesium	48 mg	12%
Phosphorus	168 mg	17%
Potassium	291 mg	8%
Zinc	1.5 gm	10%
Copper	0.4 mg	18%
Manganese	1 mg	52%
Selenium	3.7 µg	5%

% Daily values (DV) are based on a 2000 calories Diet.
DV may be higher or lower depending on your calorie needs.

Table 1.3 Nutrition facts about cooked chickpeas. Source: USDA (2020)

1.5.2 Proteins and Amino Acids Content of Chickpea

The primary proteins found in legumes, including chickpea, belong to the albumin and globulin groups. The major globulins in legumes are legumin (11S), vicilin (7S), and convicilin (15S) (Schwenke, 2001). In smaller amounts, other proteins such as gluteins and prolamines are also present in legumes like chickpeas (Gupta and Dhillon, 1993; Saharan and Khetarpaul, 1994). Prolamines, known for their high content of proline and glutamine, are alcohol-soluble, while gluteins are soluble in dilute acids, bases, detergents, and other solutions. Gluteins, which have higher concentrations of methionine and cystine compared to globulins, are considered a more important nutrient, leading some researchers to suggest focusing on increasing glutein content in leguminous crops (Singh and Jambunathan, 1982).

A study by Dhawan et al. (1991) on six chickpea varieties found that protein content ranged from 20.9% to 25.27%, with the proportions of albumin, globulin, glutein, and prolamine being 8.39–12.31%, 53.44–60.29%, 3.12–6.89%, and 19.38–24.40%, respectively. In another study (da Silva et al. 2001), chickpea proteins consisted of 41.79% globulins, 16.18% albumins, 9.99% gluteins, and 0.48% prolamines.

Chickpea protein digestibility varies widely, from 48% to 89.01%, depending on the study (Chitra et al., 1995, 1996; Clemente et al., 1998; Prakash and Prakash, 1999; Monsoor and Yusuf, 2002; Han et al., 2007). Protein digestibility can be enhanced through processes like fermentation (Frias et al., 1995). For example, digestibility of chickpea flour can improve from 72.2–83.2% to 83.7–88.8% through fermentation, which also enhances its texture, aroma, and flavor. Fermented chickpea flour has higher levels of essential amino acids like methionine, cysteine, phenylalanine, tyrosine, and threonine compared to non-fermented flour (Angulo-Bejarano et al., 2008).

Additionally, Singh and Jambunathan (1981) found that kabuli chickpea varieties (Dhal type) have higher in vitro digestibility, ranging from 72.7–79.1% for dhal and 52.4–69% for whole beans, compared to the desi varieties, which showed a range of 63.7–76% for dhal and the same digestibility (52.4–69%) for whole seeds.

Legume proteins are rich in lysine, leucine, aspartic acid, glutamic acid, and arginine, with elevated levels of branched-chain amino acids like isoleucine, leucine, and valine (Table 1.6), which are considered beneficial for health (Swanson, 1990; Oomah, 2001). Differences in the protein composition of chickpeas and other legumes are influenced by factors such as variety, environmental conditions, geographic location, growing season, and the method of analysis used (Maheri-Sis et al., 2008; Alajaji and El-Adawy, 2006; Zia-Ul-Haq et al., 2007).

Type of amino acid	Amino acid content		
	g/100g sample[a]	g/16g N[b]	g/100g protein[c]
Essential amino acids			
Isoleucine	0.36	4.1	4.5–4.8
Leucine	0.48	7.0	8.1–8.5
Lysine	0.91	7.7	6.7–7.0
Methionine	0.12	1.6	0.8–1.1
Phenylalanine	0.42	5.9	5.0–5.3
Threonine	0.06	3.6	2.7–3.0
Tryptophan	—	1.1	0.8–0.9
Valine	0.38	3.6	4.1–4.6
Cystine	—	1.3	0.4–0.6
Tyrosine	0.19	3.7	2.6–2.8
Nonessential amino acids			
Alanine	0.26	4.4	47–5.2
Arginine	0.48	10.3	8.0–8.5
Aspartic acid	0.58	11.4	10.9–11.5
Glutamic acid	1.67	17.3	17.3–17.8
Glycine	0.26	4.1	3.4–3.6
Histidine	0.24	3.4	2.9–3.2
Proline	0.24	4.6	3.8–4.1
Serine	0.12	4.9	3.3–3.7

Source: [a]Candela et al. (1997), [b]Alajaji and El-Adawy (2006), [c]Zia-Ul-Haq et al. (2007).

Table 1.4 Amino acid composition of chickpea grains.

1.5.3 Lipids and Fatty Acids

The total lipid content in chickpeas typically ranges between 4.5 and 6.0 grams of oil per one hundred grams of beans (Boye et al., 2010). Triglycerides form most of the neutral lipids, while lecithin is the primary part of polar lipids. Chickpea fat is known for its high content of essential unsaturated fatty acids, with linoleic acid being the most abundant (54.7–56.2% of the oil), followed by oleic acid (21.6–22.2%), and linolenic acid (0.5–0.9%). Additionally, smaller amounts of palmitic acid (18.9–20.4%) and stearic acid (1.3–1.7%) are also present.

Linoleic acid is particularly valuable due to its role in the body's tissue metabolism, where it aids in the production of prostaglandins—compounds that help reduce blood pressure and regulate smooth muscle contractions (Zia-Ul-Haq et al., 2007). Other components of chickpea fat include waxes, fatty alcohols, and sterols, which can be diminished through chemical processes such as flour protein isolation (Sánchez-Vioque et al., 1998).

1.5.4 Minerals

Chickpeas are a rich source of essential minerals, including calcium (Ca), phosphorus (P), magnesium (Mg), iron (Fe), and potassium (K) (Table 1.7). However, these mineral contents can decrease when chickpea grains undergo thermal processing (Wang et al., 2010; Alajaji and El-Adawy, 2006). Compared to other legumes, chickpeas are particularly high in manganese,

zinc, and phosphorus (Wang et al., 2010). These minerals are crucial for various physiological functions, including bone health, energy production, and immune system support.

	Minerals (mg/100g dm)						
Type	Ca	K	Mg	Fe	P	Zn	Mn
Desi chickpea	165.0	994.5	169.0	4.59	451.5	4.07	3.81
Kabuli chickpea	81.7	1060.0	147.0	5.50	394.0	3.40	3.28

Source: Wang et al. (2010).

Table 1.5 Mineral content of chickpea for Desi and Kabuli.

1.5.5 Carbohydrates

Chickpea seeds, in both their grain and flour forms, have significant amounts of monosaccharides, disaccharides, and oligosaccharides (Table 1.8). The primary monosaccharides found in chickpea seeds include ribose, fructose, and glucose. Additionally, they contain disaccharides like sucrose and maltose. Among the main oligosaccharides present in chickpea are raffinose, ciceritol, stachyose, and a minor amount of verbascose (Sánchez-Mata et al., 1998; Alajaji and El-Adawy, 2006).

Compounds	Chickpea grains
Monosaccharides	0.32–0.97
Ribose	0.03–0.19
Fructose	0.23–0.28
Glucose	0–0.065
Disaccharides:	
Sucrose	1.09–2.28
Maltose	0.16–0.68
Oligosaccharides:	3.87–6.98
Raffinose	0.62–1.45
Ciceritol	2.51–2.78
Stachyose	0.74–2.56
Verbascose	0–0.19

Source: Sánchez-Mata et al. (1998); Alajaji and El-Adawy (2006).

Table1.6 Carbohydrates content of chickpea seeds.

1.5.6 Antinutritional Compounds and Effect of Processing Techniques to Chickpeas

Antinutritional compounds are substances that interfere with the digestion process. The accumulation of these compounds in legume grains is thought to be an evolutionary defense mechanism against unfavorable environmental conditions, parasites, fungi, insects, and herbivores. These compounds in pulse crops are categorized into two types: protein and non-protein antinutritional components. Their effects range from relatively harmless polyphenols to more harmful protease inhibitors. Non-protein antinutritional components include alkaloids, phytic acid, oligosaccharides, and phenolic compounds like tannins and saponins. Protein-based antinutritional compounds often found in legumes include lectins (also known as agglutinins), trypsin inhibitors, chymotrypsin inhibitors, and antifungal peptides (Roy et al., 2010).

Antinutritional compounds in chickpea (Table 1.9) can be reduced through heat treatments. Lectins, which are carbohydrate-binding proteins, are widespread in plants, with several hundred diverse types found. There are four major groups of lectins: legume lectins, chitin-binding lectins, monocot mannose-binding lectins, and ribosome-inactivating proteins type 2. Legumes contain many legume lectins, but in chickpea, the agglutination activity is lower (four hundred units/g) compared to lentils and peas, and varies based on variety, growing conditions, and collection methods (Singh, 1988). Lectins have been linked to symptoms like diarrhea, bloating, vomiting, and red blood cell agglutination when raw grains or flour are consumed (Peumans and Van Damme, 1996). Despite this, research has shown that legume lectins have potential therapeutic uses in controlling obesity and reducing cancer risks (Sames et al., 2001).

Chickpea seeds contain also trypsin inhibitors (6.7–14.6 units/mg) and chymotrypsin inhibitors (5.7–94 units/mg), which hinder protein digestion, lowering the body's ability to use proteins. Chickpea amylase inhibitor content ranges from 0 to 15 units/g (Singh, 1988). Ultrafiltration and defatting processes can reduce these inhibitors, improving protein availability from 22.3% to 88.0% for desi and from 18.9% to 85.7% for kabuli seeds (Mondor et al., 2009). Extrusion techniques used in kidney beans similarly reduce protease and amylase inhibitors and cut agglutination activity while decreasing condensed tannins and polyphenols (Marzo et al., 2002).

Another antinutritional substance in chickpea is phytic acid, which forms insoluble complexes with minerals like calcium, zinc, and iron, hindering their absorption. Chickpea seeds have

lower phytic acid concentrations (4.9–6.1 mg/g) compared to other legumes like kidney beans, fava beans, and soybeans (Thavarajah et al., 2009). Other studies report even lower phytic acid levels in chickpeas (1.38–1.71 mg/g) (Zia-Ul-Haq et al., 2007). Combining chickpea flour with wheat flour reduces phytic acid content, and heating processes can further lower it by 20% (Xu and Chang, 2009; Shahzadi et al., 2007; Mondor et al., 2009).

Chickpea seeds also contain oligosaccharides such as stachyose, raffinose, and verbascose, which cause flatulence by producing gas during bacterial fermentation in the large intestine. Traditional cooking methods and microwave cooking can reduce these oligosaccharides by up to 42% (Berrios et al., 2010). Additionally, chickpea seeds are rich in polyphenols and flavonoids, which have strong antioxidant properties, particularly concentrated in the skin of the seeds. Darker-colored chickpeas tend to have higher concentrations of these compounds (Segev et al., 2010). The total polyphenolic content in chickpea ranges from 0.72 to 1.81 mg/g, with anthocyanins present at 14.9 mg/kg, depending on the extraction methods used (Xu et al., 2007; Segev et al., 2010; Silva-Cristobel et al., 2010).

Although chickpeas have lower polyphenol and anthocyanin levels compared to black beans or lentils, they remain a valuable source of phenolic acids like cinnamic, salicylic, hydroxycinnamic, p-coumaric, gallic, caffeic, vanillic, ferulic, anise, tannic, isoferulic, piperonyl, and chlorogenic acids, which are potent antioxidants that help reduce oxidative stress in the body (Tiwari et al., 2009). Chickpea seeds also contain bioactive compounds, such as isoflavones, which have antioxidant, estrogenic, antifungal, and antibacterial properties (Zhao et al., 2009).

Processing chickpea seeds can reduce antinutritional components. For example, isolating proteins from chickpea flour reduces polyphenolic content by 20%. Protein concentrates made from full-fat flour have lower polyphenol content (1.34 mg/g) than those from defatted flour (1.48 mg/g) (Mondor et al., 2009). Microwave cooking, autoclaving, and traditional cooking methods can also reduce saponins and condensed tannins, with microwave heating achieving the highest tannin reduction (50.1%). These processes also decrease trypsin inhibitor activity by over 80% (Alajaji and El-Adawy, 2006).

Antinutritional compounds	Chickpea processing					Reference
	Raw	Boiled/Cooked	Autoclaved	Microwave cooked	Dry heating	
Trypsin inhibitor activity (mg protein/dm)	11.90	2.11	1.92	2.32	—	Alajaji and El-Adawy (2006)
Trypsin inhibitor activity TIA (mg/g dm)	8.29	0.75	—	—	—	Wang et al. (2010)
Phytic acid (mg/g)	1.21	0.86	0.71	0.75	—	Alajaji and El-Adawy (2006)
Phytic acid (g/kg)	10.6	11.2	—	—	—	Wang et al. (2010)
Polyphenols	3.39	1.35	—	—	—	Attia et al. (1994)
Saponin (mg/g)	0.91	0.44	0.51	0.48	—	Alajaji and El-Adawy (2006)
Tannins (mg/g)	4.85	2.52	2.42	2.50	—	Alajaji and El-Adawy (2006)
Total carbohydrates (g/100g dm)	56.21	—	—	—	42.51	Frias et al. (2000)

Table 1.7 Antinutritional compounds in chickpea seeds. Source: Alajaji and EI-Adawy (2006)

1.6 METHODS OF PROTEIN DETERMINATION

The Kjeldahl method, one of the oldest and most widely used techniques, measures the total nitrogen content to estimate protein levels, assuming protein contains 16% of nitrogen. This method involves digesting the sample with concentrated sulfuric acid to convert organic nitrogen to ammonium sulfate, followed by distillation and titration of the resulting ammonia. While reliable and reproducible, the Kjeldahl method also quantifies non-protein nitrogen, which can lead to overestimated protein values (Haug & Lantzsch, 1983). Additionally, the method is time-intensive and involves managing hazardous acids.

Another important method is the Dumas combustion method that determines the protein content of chickpeas by measuring their nitrogen content. A finely ground sample is combusted at high temperatures (800–1,000°C) in an oxygen-rich environment, converting all nitrogen into nitrogen gas (N2_22). This nitrogen is then separated from other gases and quantified using a thermal conductivity detector. The percentage of nitrogen in the sample is calculated and multiplied by a conversion factor (commonly N×6.25N \times 6.25N×6.25) to estimate the protein content, as nitrogen is a key component of proteins. This method is fast, reliable, and widely used in food analysis for its precision.

The Bradford assay is a simpler and faster spectrophotometric method that detects proteins based on the binding of Coomassie Brilliant Blue dye. The dye's color intensity shifts in proportion to the protein concentration, enabling quantification via a standard curve. This

method is valued for its speed and sensitivity across various protein concentrations, but it may be affected by the chemical properties of the proteins or the presence of interfering substances, like detergents (Bradford et al., 1976).

The Lowry method, a combination of the biuret reaction and the Folin-Ciocalteu reagent's reduction, is known for its high sensitivity, making it suitable for detecting proteins at lower concentrations. However, its complexity and longer reaction times, along with interference from compounds like phenolics and sugars, can pose practical challenges (Lowry et al., 1951). Despite these limitations, the Lowry method is widely used in research for measuring protein levels in chickpea seeds, emphasizing careful sample preparation.

The Bicinchoninic Acid (BCA) assay, another colorimetric method, is like the Lowry method but offers improved stability and linearity. In this assay, bicinchoninic acid forms a colored complex with cuprous ions generated by protein-induced copper ion reduction. The BCA method is helpful due to its compatibility with a range of buffers and detergents, making it more versatile than some other methods (Smith et al., 1985). However, like other assays, it can still be influenced by interfering substances in chickpea extracts, needing proper sample handling and calibration.

1.7 GENETIC TOOLS FOR THE CHARACTERIZATION OF CHICKPEA GENETIC RESOURCES

Understanding the genetic diversity of chickpea germplasm is crucial for improving traits such as yield, disease resistance, and tolerance to abiotic stresses. Genetic diversity within chickpea populations is a key resource for breeding programs. Wide sets of materials can be evaluated by using both phenotypic and genotypic tools.

The development of high-throughput sequencing technologies allows the genomic characterization of wide sets of genetic resources, while the application of population genetics approaches, and genome-wide analysis allows the identification of the genetic control of phenotypic variance for specific traits. In the last decade, such methods have been largely used for chickpea genetic resources, which created a reference set of genomics data useful for the scientific community and plant breeders (Roorkiwal et al., 2020).

Three reference genomes are available for chickpea: one for the kabuli type (CDC Frontier genotype; Varshney et al., 2013), one for the desi type (ICC 4958 genotype; Parween et al.,

2015), and one for a wild *C. reticulatum* accession (PI489777; Gupta et al., 2017). The three genomes, together with *de novo* whole-genome sequencing data (aligned to CDC Frontier) from 3,171 cultivated and 28 *C. reticulatum* accessions, were used to guide the assembly of the chickpea pan-genome (Varshney et al., 2021). Several works are already published on genomic characterization of chickpea diverse sets of materials. Thudi et al. (2016) shows the whole-genome sequencing analysis of a set of 129 commercial chickpea varieties that were released between 1948 and 2012 in 14 countries. Resequencing data are also available for a panel of a diverse set of 429 lines that were collected in 45 countries (Varshney et al., 2019).

Availability of genomic and phenotypic data allows the identification of Quantitative Traits Loci (QTL) associated to the trait of interest by applying linkage or association mapping methods. Such genomic information facilitates marker-assisted selection (MAS), allowing breeders to use markers associated with desirable traits to accelerate the identification of superior genotypes, as well as genomic selection.

Studies have revealed many QTLs linked to traits such as seed size, maturation timing, and stress responses. Notably, significant QTLs for drought tolerance and *Ascochyta* blight resistance have been identified, offering critical insights for breeding programs aimed at enhancing chickpea resilience in diverse environmental conditions (Mandal et al., 2021). This targeted breeding approach improves efficiency and helps ensure that new cultivars are better adapted to specific climates and resistant to prevalent diseases.

Moreover, genomic selection is reshaping chickpea breeding practices. This technique uses genome-wide markers to predict the breeding value of individuals based on their genetic makeup, enabling breeders to make more informed decisions during the selection process. By incorporating genomic data, breeders can more effectively select for complex polygenic traits, accelerating genetic improvements compared to traditional breeding methods (Jiang & Zhang, 2019).

1.8 GENOTYPE BY ENVIRONMENT INTERACTION

1.8.1 Understanding Genotype by Environment Interaction

Genotype by environment interaction (GEI) is a critical concept in plant breeding and agronomy, particularly for chickpea (*Cicer arietinum* L.). GEI refers to the varying performance of genotypes across different environmental conditions. Understanding GEI is

essential for developing chickpea varieties that are high-yielding and resilient across diverse climates. Studies have shown that GEI has a notable impact on yield stability, with some genotypes performing better in specific environments (Gauch & Zobel, 1996).

Breeding programs targeting GEI insights allow for improved adaptability to environmental stresses like drought, heat, and soil nutrient limitations. Research has revealed that certain chickpea genotypes show superior drought tolerance, producing higher yields in water-scarce conditions (Khan et al., 2017). This highlights the importance of selecting genotypes that are suited to the environmental challenges of specific regions, such as those prone to drought *versus* regions where yield potential and disease resistance are prioritized (Shah et al., 2018).

Temperature and soil fertility also significantly influence GEI in chickpea. Temperature variations can affect growth, with some genotypes performing better under higher temperatures (Bhaumik et al., 2018). Likewise, nutrient availability in soils affects chickpea yields, emphasizing the importance of regional soil management practices (Kumar et al., 2020).

Multi-environment trials (MET) are an effective way to incorporate GEI knowledge into breeding strategies, as they evaluate varieties across various environmental conditions. METs help breeders identify stable, high-performing genotypes that can thrive in specific climates. Advances in molecular techniques, such as marker-assisted and genomic selection, now allow for more targeted approaches to managing GEI, making breeding programs more efficient (Yadav et al., 2021). Additionally, participatory breeding, which involves local farmers in the selection process, strengthens these programs by aligning them with region-specific needs and preferences (Manickavelu et al., 2019).

1.8.2 Significance of GEI in Chickpea.

With increasing climate variability, selecting genotypes that consistently maintain yields across different environments is key to improving food security and production (Khan et al., 2017). Studies have shown that some chickpea genotypes demonstrate higher drought resilience, maintaining better yields under water-limited conditions (Bhaumik et al., 2018). This understanding allows breeders to focus on developing varieties that can withstand abiotic stresses, leading to increased productivity in areas vulnerable to climate changes (Moumeni et al., 2016).

GEI also guides the design of METs, which are essential for evaluating genotype performance across different regions. These trials enable the identification of stable genotypes, providing breeders with valuable data for selecting varieties most suitable for specific environmental conditions (Crossa et al., 2017). Through data from METs, breeders can improve selection criteria and enhance genetic gains, ensuring that new varieties meet both environmental challenges and the needs of local farmers (Manickavelu et al., 2019).

1.8.3 Environmental Factors Impacting Chickpea Protein Content and GEI

Environmental factors such as moisture availability, temperature, and soil fertility play crucial roles in determining seed protein content and genotype-environment interaction (GEI) in chickpea. Moisture availability during the growing season is a primary factor affecting seed protein levels. Drought stress, particularly during the reproductive phase, tends to increase protein concentration as an adaptive response to water scarcity (Nazar et al., 2019). However, this increase typically comes at the expense of yield, highlighting a trade-off between seed quality and quantity. Effective water management is crucial for balancing protein content with adequate yield.

Temperature significantly impacts protein synthesis in chickpea. Elevated temperatures during key stages, such as flowering and seed filling, can reduce protein yield by decreasing photosynthetic efficiency and increasing respiration (Jagadish et al., 2015). In contrast, moderate temperatures during these stages improve protein synthesis, enhancing seed nutritional quality. Soil fertility, especially the availability of nitrogen and phosphorus, is another critical factor for seed protein content. Chickpea form a symbiotic relationship with *Rhizobium* bacteria, which helps fix atmospheric nitrogen, contributing to higher seed protein levels. However, nutrient deficiencies, as well as factors like soil pH and organic matter content, can limit nutrient uptake, reducing protein concentration (Kumar et al., 2021).

Soil characteristics, such as nutrient availability and water retention, vary across regions, and chickpea genotypes may respond differently to these conditions. Some perform well in nutrient-rich soils, while others struggle in less fertile environments (Kumar et al., 2020). Water availability, particularly in drought-prone areas, is also critical. Drought-tolerant genotypes maintain higher yields under water-limited conditions, making selection of such genotypes vital for arid regions (Bhaumik et al., 2018). Temperature plays a crucial role in GEI, with rising global temperatures negatively affecting flowering and seed set, leading to

lower yields (Yadav et al., 2021). Breeding heat-resistant varieties is necessary to ensure productivity as climate change intensifies. Other atmospheric conditions, such as humidity and light intensity, also influence physiological processes like transpiration and photosynthesis, further affecting growth and yield (Shah et al., 2018). Breeding programs focused on drought tolerance, temperature resilience, and improved nutrient use efficiency can develop cultivars that maintain high protein levels even under adverse conditions (Khan et al., 2019).

1.8.4 Methods to Measure Genotype by Environment Interaction

One of the most widely used methods for measuring GEI is Analysis of Variance (ANOVA) for a mixed model, which partitions variance among genotypes, environments, and their interactions. This method helps researchers to compare genotype performance across different environments, identifying stable genotypes that perform consistently despite environmental variability (Patterson & Thompson, 1971).

Advanced statistical models, such as the additive main effects and multiplicative interaction (AMMI) model, are often applied to MET data to separate genotype and environmental main effects from their interaction (Gauch, 2006). This enhances breeders' ability to predict genotype performance in various environments, supporting the development of climate-resilient crops. Genomic approaches like genome-wide association studies (GWAS) are increasingly being used to measure GEI (Mackay et al., 2009). GWAS helps to identify genetic markers associated with traits that perform well in different environments (Huang et al., 2015). By correlating genetic data with phenotypic outcomes across various environments, breeders can develop molecular markers for selecting genotypes suited to specific environmental conditions.

Machine learning techniques, such as random forests and support vector machines, are powerful tools for modeling GEI interactions and predicting genotype performance (Heslot et al., 2012). These methods have been successfully applied in GEI studies to analyze data from METs and genomic analyses. For example, random forests have been used to rank the importance of environmental variables in determining genotype performance, enabling researchers to identify key factors driving GEI (Sandhu et al., 2021). Support vector machines, on the other hand, have been utilized to predict yield stability across different environments by modeling complex interactions between genetic markers and environmental conditions (Huang et al., 2019). These applications provide deeper insights into genotype stability and

adaptability, allowing for the selection of genotypes better suited to specific environmental stresses (Lebdi et al., 2020).

1.9 RESEARCH OBJECTIVES

In the present study we aim to:

- investigate variability for crude protein content in a wide set of chickpeas materials;
- understand how genotype, environment and their interaction effects contribute to variability in crude protein content;
- determine the heritability of crude protein content;
- identify high performing genotypes for this nutritional trait.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL SELECTION

In this study, the European and Mediterranean Chickpea Association Panel, EMCAP (Rocchetti et al. 2020), was used. The EMCAP panel consists of 480 genotypes derived through single-seed descent (SSD) from worldwide collections mainly maintained in genebanks. The majority of the lines were derived from landraces that capture the genetic diversity of domesticated chickpeas worldwide, with an emphasis on European and Mediterranean environments. The EMCAP panel was phenotypically evaluated in multi-year field trials conducted at the CREA-CI experimental station in Osimo, Ancona, Italy ((latitude 43.463794, longitude 13.496916), over three growing seasons: 2019, 2020, and 2021. The seeds harvested in the 2019 and 2021 trials were used in the present study. Daily weather data, including average, maximum, and minimum temperatures (°C) as well as rainfall (mm), were recorded using field-based weather stations. Thermo-pluviometric graphs (Figure 2.1) summarize the average monthly temperature and rainfall for 2019 and 2020 years.

A Randomized Block Design (RBD) was employed, with two replicates in 2019 and three replicates in 2020 and 2021. Each experimental unit was comprised of a single-row plot containing 10 seeds, sown with a 10 cm spacing between seeds. Sowing was carried out manually in early April (spring-summer growth cycle). Weed control was maintained manually throughout the growth period to ensure minimal competition. At physiological maturity, plants were harvested by uprooting and allowed to air-dry in the field before being threshed using a specialized research plot harvester. From the initial set of 480 genotypes, 202 lines from the 2019 and 2021 growing season were selected for in-depth analysis in this study. Details on the origin, biological status, and market type of these selected lines are provided in Table 2.1.

Table 2.1 List of plant materials selected for this study.

Accession/ line code	Species	Country ISO code	Biological status	Desi/Kabuli
AN_Ca_0004	*Cicer arietinum*	ALB	Landrace	Desi
AN_Ca_0009	*Cicer arietinum*	BGR	Breeding material	Desi
AN_Ca_0015	*Cicer arietinum*	EGY	Landrace	Desi
AN_Ca_0020	*Cicer arietinum*	GRC	Landrace	Kabuli
AN_Ca_0021	*Cicer arietinum*	GRC	Landrace	Kabuli
AN_Ca_0024	*Cicer arietinum*	GRC	Landrace	Kabuli

AN_Ca_0026	*Cicer arietinum*	GRC	Landrace	Desi
AN_Ca_0027	*Cicer arietinum*	GRC	Landrace	Desi
AN_Ca_0028	*Cicer arietinum*	GRC	Landrace	Desi
AN_Ca_0031	*Cicer arietinum*	GRC	Landrace	Desi
AN_Ca_0035	*Cicer arietinum*	ITA	Landrace	Desi
AN_Ca_0039	*Cicer arietinum*	ITA	Landrace	Desi
AN_Ca_0040	*Cicer arietinum*	ITA	Landrace	Desi
AN_Ca_0048	*Cicer arietinum*	ITA	Landrace	Kabuli
AN_Ca_0049	*Cicer arietinum*	ITA	Landrace	Kabuli
AN_Ca_0052	*Cicer arietinum*	ITA	Landrace	Kabuli
AN_Ca_0053	*Cicer arietinum*	ITA	Landrace	Desi
AN_Ca_0063	*Cicer arietinum*	ITA	Landrace	Kabuli
AN_Ca_0073	*Cicer arietinum*	ITA	Landrace	Kabuli
AN_Ca_0078	*Cicer arietinum*	ITA	Landrace	Kabuli
AN_Ca_0086	*Cicer arietinum*	ITA	Landrace	Kabuli
AN_Ca_0092	*Cicer arietinum*	ITA	Landrace	Desi
AN_Ca_0097	*Cicer arietinum*	ITA	Landrace	Desi
AN_Ca_0107	*Cicer arietinum*	ITA	Landrace	Desi
AN_Ca_0110	*Cicer arietinum*	ITA	Landrace	Desi
AN_Ca_0115	*Cicer arietinum*	ITA	Landrace	Kabuli
AN_Ca_0119	*Cicer arietinum*	ITA	Landrace	Kabuli
AN_Ca_0126	*Cicer arietinum*	ITA	Landrace	Kabuli
AN_Ca_0129	*Cicer arietinum*	ITA	Landrace	Desi
AN_Ca_0131	*Cicer arietinum*	ITA	Landrace	Kabuli
AN_Ca_0136	*Cicer arietinum*	ITA	Landrace	Kabuli
AN_Ca_0153	*Cicer arietinum*	ITA	Landrace	Desi
AN_Ca_0158	*Cicer arietinum*	ITA	Breeding material	Desi
AN_Ca_0159	*Cicer arietinum*	ITA	Landrace	Kabuli
AN_Ca_0160	*Cicer arietinum*	ITA	Landrace	Kabuli
AN_Ca_0169	*Cicer arietinum*	SVK	Landrace	Kabuli
AN_Ca_0175	*Cicer arietinum*	TUN	Landrace	Desi
AN_Ca_0195	*Cicer arietinum*	TUR	Landrace	Kabuli
AN_Ca_0197	*Cicer arietinum*	TUR	Landrace	Desi
AN_Ca_0201	*Cicer arietinum*	PAK	Landrace	Desi
AN_Ca_0211	*Cicer arietinum*		Landrace	Kabuli
AN_Ca_0219	*Cicer arietinum*	NPL	Landrace	Desi
AN_Ca_0224	*Cicer arietinum*	TJK	Landrace	Desi
AN_Ca_0226	*Cicer arietinum*	TJK	Landrace	Desi
AN_Ca_0234	*Cicer arietinum*	ESP	Landrace	Desi
AN_Ca_0238	*Cicer arietinum*	BGR	Landrace	Desi
AN_Ca_0249	*Cicer arietinum*	ROU	Landrace	Desi
AN_Ca_0250	*Cicer arietinum*	HUN	Landrace	Desi
AN_Ca_0251	*Cicer arietinum*	DEU	Landrace	Desi

AN_Ca_0252	*Cicer arietinum*	DEU	Landrace	Desi
AN_Ca_0253	*Cicer arietinum*	DEU	Landrace	Desi
AN_Ca_0255	*Cicer arietinum*	UKR	Cultivar	Desi
AN_Ca_0256	*Cicer arietinum*	UKR	Cultivar	Desi
AN_Ca_0283	*Cicer arietinum*	MAR	Landrace	Desi
AN_Ca_0290	*Cicer arietinum*	AFG	Landrace	Desi
AN_Ca_0292	*Cicer arietinum*	AFG	Landrace	Desi
AN_Ca_0293	*Cicer arietinum*	AFG	Landrace	Desi
AN_Ca_0315	*Cicer arietinum*	MKD	Cultivar	Desi
AN_Ca_0317	*Cicer arietinum*	MKD	Cultivar	Desi
AN_Ca_0320	*Cicer arietinum*	AFG	Landrace	Desi
AN_Ca_0322	*Cicer arietinum*	AFG	Landrace	Desi
AN_Ca_0334	*Cicer arietinum*	ESP	Cultivar	Desi
AN_Ca_0348	*Cicer arietinum*	ESP	Cultivar	Desi
AN_Ca_0356	*Cicer arietinum*	TJK	Landrace	Desi
AN_Ca_0358	*Cicer arietinum*	IND	Cultivar	Desi
AN_Ca_0370	*Cicer arietinum*	ITA	Cultivar	Kabuli
AN_Ca_0372	*Cicer arietinum*	ITA	Breeding material	Kabuli
AN_Ca_0377	*Cicer arietinum*	ITA	Breeding material	Desi
AN_Ca_0380	*Cicer arietinum*	IRQ	Landrace	Desi
AN_Ca_0390	*Cicer arietinum*	IND	Landrace	Desi
AN_Ca_0398	*Cicer arietinum*	SYR	Landrace	Desi
AN_Ca_0404	*Cicer arietinum*	IRQ	Landrace	Desi
AN_Ca_0409	*Cicer arietinum*	ITA	Landrace	Desi
AN_Ca_0410	*Cicer arietinum*	MEX	Cultivar	Desi
AN_Ca_0418	*Cicer arietinum*	PAK	Cultivar	Desi
AN_Ca_0419	*Cicer arietinum*	PAK	Landrace	Desi
AN_Ca_0421	*Cicer arietinum*	PAK	Cultivar	Desi
AN_Ca_0448	*Cicer arietinum*	TUR	Landrace	Desi
AN_Ca_0449	*Cicer arietinum*	TUR	Landrace	Desi
AN_Ca_0452	*Cicer arietinum*	TUR	Landrace	Desi
AN_Ca_0456	*Cicer arietinum*	TUR	Landrace	Desi
AN_Ca_0457	*Cicer arietinum*	TUR	Landrace	Desi
AN_Ca_0458	*Cicer arietinum*	TUR	Landrace	Desi
AN_Ca_0460	*Cicer arietinum*	TUR	Landrace	Desi
AN_Ca_0467	*Cicer arietinum*	TUR	Landrace	Desi
AN_Ca_0500	*Cicer arietinum*	TUR	Landrace	Desi
AN_Ca_0501	*Cicer arietinum*	TUR	Landrace	Desi
AN_Ca_0513	*Cicer arietinum*	TUR	Landrace	Desi
AN_Ca_0519	*Cicer arietinum*	TUR	Landrace	Desi
AN_Ca_0531	*Cicer arietinum*	USA	Landrace	Kabuli
AN_Ca_0561	*Cicer arietinum*	PAK	Breeding material	Desi
AN_Ca_0562	*Cicer arietinum*	PAK	Landrace	Desi

AN_Ca_0563	*Cicer arietinum*	PAK	Breeding material	Desi
AN_Ca_0564	*Cicer arietinum*	ESP	Landrace	Desi
AN_Ca_0567	*Cicer arietinum*	PAK	Landrace	Desi
AN_Ca_0571	*Cicer arietinum*	CHN	Landrace	Desi
AN_Ca_0572	*Cicer arietinum*	ETH	Landrace	Desi
AN_Ca_0574	*Cicer arietinum*	ETH	Landrace	Desi
AN_Ca_0578	*Cicer arietinum*	ETH	Landrace	Desi
AN_Ca_0580	*Cicer arietinum*	ETH	Landrace	Desi
AN_Ca_0582	*Cicer arietinum*	ETH	Landrace	Desi
AN_Ca_0583	*Cicer arietinum*	ETH	Landrace	Desi
AN_Ca_0584	*Cicer arietinum*	ETH	Landrace	Desi
AN_Ca_0586	*Cicer arietinum*	ETH	Landrace	Desi
AN_Ca_0587	*Cicer arietinum*	ETH	Landrace	Desi
AN_Ca_0588	*Cicer arietinum*	ETH	Landrace	Desi
AN_Ca_0589	*Cicer arietinum*	ETH	Landrace	Desi
AN_Ca_0593	*Cicer arietinum*	ETH	Landrace	Desi
AN_Ca_0594	*Cicer arietinum*	ETH	Landrace	Desi
AN_Ca_0596	*Cicer arietinum*	ETH	Landrace	Desi
AN_Ca_0597	*Cicer arietinum*	PAK	Landrace	Desi
AN_Ca_0599	*Cicer arietinum*	BGD	Cultivar	Desi
AN_Ca_0600	*Cicer arietinum*	BGD	Cultivar	Desi
AN_Ca_0601	*Cicer arietinum*	BGD	Cultivar	Desi
AN_Ca_0604	*Cicer arietinum*	UZB		Desi
AN_Ca_0618	*Cicer arietinum*	MDA	Landrace	Kabuli
AN_Ca_0620	*Cicer arietinum*	AUS	Cultivar	Desi
AN_Ca_0621	*Cicer arietinum*	IND	Breeding material	Desi
AN_Ca_0623	*Cicer arietinum*	USA	Landrace	Kabuli
AN_Ca_0643	*Cicer arietinum*	ARM		Desi
AN_Ca_0646	*Cicer arietinum*	ESP	Breeding material	Kabuli
AN_Ca_0658	*Cicer arietinum*	TJK	Landrace	Desi
AN_Ca_0659	*Cicer arietinum*	TJK	Landrace	Desi
AN_Ca_0660	*Cicer arietinum*	TJK	Landrace	Desi
AN_Ca_0662	*Cicer arietinum*	TJK	Landrace	Desi
AN_Ca_0666	*Cicer arietinum*	IND	Landrace	Desi
AN_Ca_0674	*Cicer arietinum*	IND	Landrace	Kabuli
AN_Ca_0700	*Cicer arietinum*	IND	Landrace	Desi
AN_Ca_0704	*Cicer arietinum*	IND	Landrace	Kabuli
AN_Ca_0708	*Cicer arietinum*	IND	Landrace	Desi
AN_Ca_0713	*Cicer arietinum*	IND	Landrace	Desi
AN_Ca_0719	*Cicer arietinum*	IND	Landrace	Desi
AN_Ca_0755	*Cicer arietinum*	IND	Landrace	Desi
AN_Ca_0763	*Cicer arietinum*	IND	Landrace	Desi
AN_Ca_0766	*Cicer arietinum*	IND	Landrace	Desi

AN_Ca_0769	*Cicer arietinum*	IND	Landrace	Desi
AN_Ca_0775	*Cicer arietinum*	IND	Landrace	Desi
AN_Ca_0776	*Cicer arietinum*	IND	Landrace	Desi
AN_Ca_0777	*Cicer arietinum*	IND	Landrace	Desi
AN_Ca_0778	*Cicer arietinum*	IND	Landrace	Desi
AN_Ca_0780	*Cicer arietinum*	IND	Landrace	Desi
AN_Ca_0782	*Cicer arietinum*	IND	Landrace	Desi
AN_Ca_0783	*Cicer arietinum*	IND	Landrace	Desi
AN_Ca_0793	*Cicer arietinum*	IND	Landrace	Desi
AN_Ca_0807	*Cicer arietinum*	IND	Landrace	Desi
AN_Ca_0816	*Cicer arietinum*	IND	Landrace	Desi
AN_Ca_0820	*Cicer arietinum*	IND	Landrace	Desi
AN_Ca_0821	*Cicer arietinum*	IND	Landrace	Desi
AN_Ca_0844	*Cicer arietinum*	GEO	Landrace	Kabuli
AN_Ca_0847	*Cicer arietinum*	TJK	Landrace	Desi
AN_Ca_0850	*Cicer arietinum*	TJK	Landrace	Desi
AN_Ca_0858	*Cicer arietinum*	TJK	Landrace	Desi
AN_Ca_0861	*Cicer arietinum*	TJK	Landrace	Desi
AN_Ca_0864	*Cicer arietinum*	TJK	Landrace	Desi
AN_Ca_0871	*Cicer arietinum*	TJK	Landrace	Desi
AN_Ca_0879	*Cicer arietinum*	TJK	Landrace	Kabuli
AN_Ca_0883	*Cicer arietinum*	TJK	Landrace	Desi
AN_Ca_0884	*Cicer arietinum*	TJK	Landrace	Desi
AN_Ca_0886	*Cicer arietinum*	TJK	Landrace	Desi
AN_Ca_0887	*Cicer arietinum*	TJK	Landrace	Desi
AN_Ca_0940	*Cicer arietinum*	MAR	Landrace	Kabuli
AN_Ca_1074	*Cicer arietinum*	IRN	Landrace	Kabuli
AN_Ca_1111	*Cicer arietinum*	CHL	Landrace	Kabuli
AN_Ca_1140	*Cicer arietinum*	JOR	Landrace	Desi
AN_Ca_1343	*Cicer arietinum*	CHL	Landrace	Kabuli
AN_Ca_1353	*Cicer arietinum*	TUR	Landrace	Kabuli
AN_Ca_1364	*Cicer arietinum*	MAR	Landrace	Kabuli
AN_Ca_1394	*Cicer arietinum*	TUN	Landrace	Kabuli
AN_Ca_1495	*Cicer arietinum*	SYR	Landrace	Kabuli
AN_Ca_1595	*Cicer arietinum*	CYP	Landrace	Kabuli
AN_Ca_1598	*Cicer arietinum*	CYP	Landrace	Kabuli
AN_Ca_1686	*Cicer arietinum*	MAR	Landrace	Kabuli
AN_Ca_1778	*Cicer arietinum*	DZA	Landrace	Kabuli
AN_Ca_1786	*Cicer arietinum*	SYR	Landrace	Kabuli
AN_Ca_1958	*Cicer arietinum*	SYR	Landrace	Kabuli
AN_Ca_1964	*Cicer arietinum*	ITA	Landrace	Kabuli
AN_Ca_1966	*Cicer arietinum*	ITA	Landrace	Kabuli
AN_Ca_2032	*Cicer arietinum*	SYR	Landrace	Kabuli

AN_Ca_2034	*Cicer arietinum*	SYR	Landrace	Kabuli
AN_Ca_2090	*Cicer arietinum*	PRT	Landrace	Kabuli
AN_Ca_2091	*Cicer arietinum*	PRT	Landrace	Kabuli
AN_Ca_2108	*Cicer arietinum*		Cultivar	Kabuli
AN_Ca_2110	*Cicer arietinum*		Cultivar	Kabuli
AN_Ca_2113	*Cicer arietinum*	ITA	Landrace	Kabuli
AN_Ca_2116	*Cicer arietinum*	ITA	Landrace	Desi
AN_Ca_2118	*Cicer arietinum*	ITA	Landrace	Desi
AN_Ca_2121	*Cicer arietinum*	ITA	Landrace	Kabuli
AN_Ca_2132	*Cicer arietinum*		Breeding material	Kabuli
AN_Ca_2142	*Cicer arietinum*		Breeding material	Kabuli
AN_Ca_2144	*Cicer arietinum*		Breeding material	Kabuli
AN_Ca_2148	*Cicer arietinum*		Landrace	Kabuli
AN_Ca_2157	*Cicer arietinum*		Breeding material	Kabuli
AN_Ca_2158	*Cicer arietinum*		Breeding material	Kabuli
AN_Ca_2159	*Cicer arietinum*		Breeding material	Kabuli

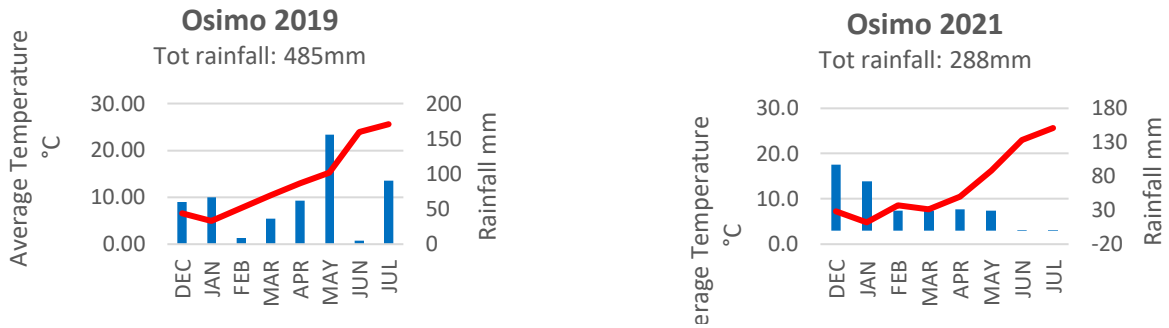

Figure 2.1 Graph shows the average rainfall pattern and temperature recorded for the various environments.

2.2 SAMPLE PREPARATION

Approximately 10 g of clean chickpea seeds were ground into a fine powder using a Retsch MM 400 instrument, which is a high-performance mixer mill commonly used in laboratories for homogenizing, pulverizing, and mixing small samples. Chickpeas samples were sealed and used for Carbon (C), Hydrogen (H), and Nitrogen (N) content determination by using the Dumas combustion method by Leco TruSpec CHN micro (Leco Corporation, St. Joseph, MI, USA). For each trial, two replicates were analysed, for a total of 388 samples.

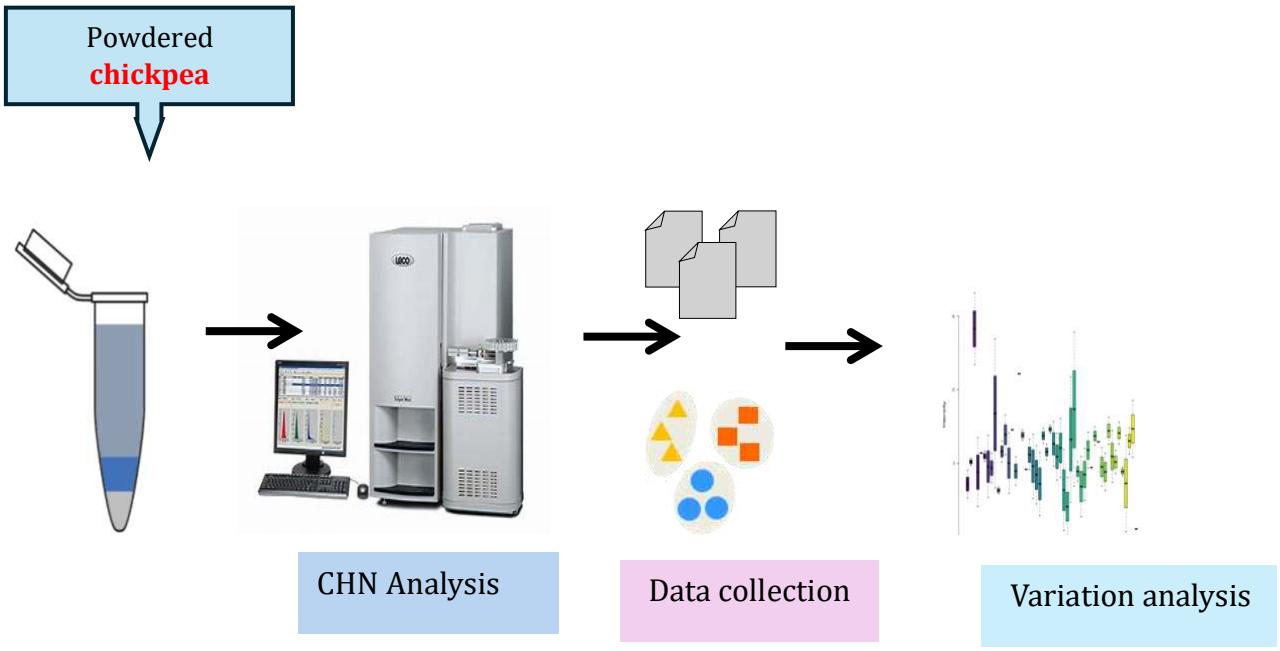

Figure 2.2 Flow diagram of crude protein measurement process

2.3 CRUDE PROTEIN DETERMINATION

Nitrogen concentration and content are commonly used as reliable indicators of the total protein content in seeds to determine the overall protein content of food products. The Jones Factor, established by D.B. Jones, is a standardized coefficient used for this purpose. The Association of Official Analytical Collaboration (AOAC) recognizes this coefficient, which is set at 6.25. Using this coefficient, the seed protein content can be calculated as follows: Seed protein content (%) = N × 6.25

2.3 STATISTICAL ANALYSIS

To analyze the data across different environments, we conducted descriptive statistics using the mean values of replicates within each environment, processed through JMP® Version 17 (SAS Institute Inc., Cary, NC, 1989–2021). This approach allowed us to capture central tendencies and variability for crude protein content across the study environments, providing a foundational understanding of environmental influences on chickpea genotypes.

To assess the heritability of protein content, we applied a linear model, where the phenotypic value (P) of a trait for any given individual in each environment is represented as the combined effect of genetic (G), environmental (E), genotype-by-environment interaction (GEI), and residual (e) components. The linear model is expressed as follows:

(1) $P = \mu + G + E + GEI + e$

where:

- P is the phenotypic value,
- μ represents the overall mean,
- G is the genetic contribution,
- E denotes environmental effects,
- GEI is the genotype-environment interaction, and
- *e* is the residual error within each environment.

To estimate the variance components for each factor (G, E, GEI) and their associated standard errors, we used the Residual Maximum Likelihood (REML) method, treating each component as a random effect. REML is advantageous here as it provides unbiased estimates of variance components, even with unbalanced data. This estimation enabled us to quantify the influence of genetics, environment, and their interaction on crude protein content.

From the calculated variance components, we estimated the broad-sense heritability (H^2) of protein content using the following formula:

(2) $H^2 = \sigma^2G / (\sigma^2G + (\sigma^2GEI/n) + (\sigma^2e/n*r)$

where σ^2G represents the genetic variance, σ^2GEI the variance of genotype-environment interaction, *n* the number of environments and *r* is the number of replicates in each environment.

The ANOVA (Analysis of Variance) framework was applied based on this linear model to test the statistical significance of genetic, environmental, and GEI effects, treating them as fixed factors in this phase of the analysis. Significance was evaluated through Tukey's post hoc test at a probability level of $p<0.05$, allowing us to identify statistically significant differences among genotypes, environments, and years.

To further classify genotypic responses, we adopted a grouping methodology initially introduced by Francis and Kannenberg (1978) and later adapted in studies like Beres et al. (2010), Gan et al. (2009), and May et al. (2010). This method involved calculating the means and coefficient of variation (CV) for each genotype and plotting them in a biplot. The biplot was divided into four categories based on the means and CV of all genotypes:

Group I: High Mean, Low Variability – Genotypes in this category displayed consistently high crude protein content across environments, indicating strong genetic stability.

Group II: High Mean, High Variability – This group consisted of genotypes with high protein content but significant sensitivity to environmental changes.

Group III (Poor): Low Mean, High Variability – Genotypes here showed both low protein content and high variability, suggesting poor adaptation and high environmental sensitivity.

Group IV (Very Poor): Low Mean, Low Variability – These genotypes had low protein content but maintained stable performance, indicating consistency at lower protein levels.

This classification provides valuable insights for breeding, as genotypes in Group I are most desirable for their stability and high performance across varied environmental conditions, while those in Groups III and IV may be more adapted to specific agro-environmental conditions.

3 RESULTS

3.1.1 DESCRIPTIVE STATISTICS FOR VARIOUS ENVIRONMENTS

Table 3.1 summarizes the crude protein content distribution across chickpea genotypes analyzed within different environmental conditions: Osimo_2019, Osimo_2021, and a combined genotype-environment interaction (GEI) dataset spanning both years. Descriptive statistics, including mean, standard deviation (SD), minimum (Min), maximum (Max), range, and coefficient of variation (CV), provide insights into the variation across these datasets. For each environment, replicate measurements were averaged per genotype to ensure robust estimates and facilitate distributional comparisons of crude protein content across the Osimo_2019 and Osimo_2021 environments. For the combined GEI dataset, average replicate values were computed across environments, allowing for an evaluation of protein content under genotype-by-environment interactions.

The Osimo_2019 dataset displayed notable variability in crude protein content, with values ranging from 7.47% to 28.67%, with a mean of 20.59%, and a range of 21.16%; this result indicates substantial diversity for protein content in the panel for the 2019 trial. The seeds harvested during the Osimo_2021 trial showed a narrower crude protein content variability, ranging from 13.63% to 27.47%, with a mean of 19.34%, and a range of 13.84%, suggesting more uniform distribution among genotypes for this trial. The combined data for both trial analyses produced a mean protein content of 19.97%, with a range similar to those of Osimo_2019, indicating that genotype-by-environment interactions did not exceed the individual environment variabilities. This data provides an understanding of environmental influence on crude protein expression across diverse genotypes and years.

Table 3.1 Descriptive statistics for crude protein content in chickpea genotypes across various environments.

Environment/Year	Mean	Std.Dev	Min	Max	Range	CV
Osimo_2019	20.59	±2.21	7.49	28.66	21.16	10.75
Osimo_2021	19.34	±2.24	13.63	27.47	13.84	11.59
GEI_2019/2021	19.97	±2.31	7.49	28.66	21.16	11.58

Figures 3.1, 3.2, and 3.3 show the frequency distribution for crude protein content in Osimo_2019, Osimo_2021, and the combined dataset. The 2019 data shows a higher frequency for protein content around 20-22% (Figure 3.1). For the 2021 data, a broader distribution was detected, with major frequency being at 18%-20% of protein content (Figure 3.2). Considering data from both trials, the frequencies were higher for classes around 19%-21% (Figure 3.3). We observed a slight shift towards lower protein percentages in the 2021 trial.

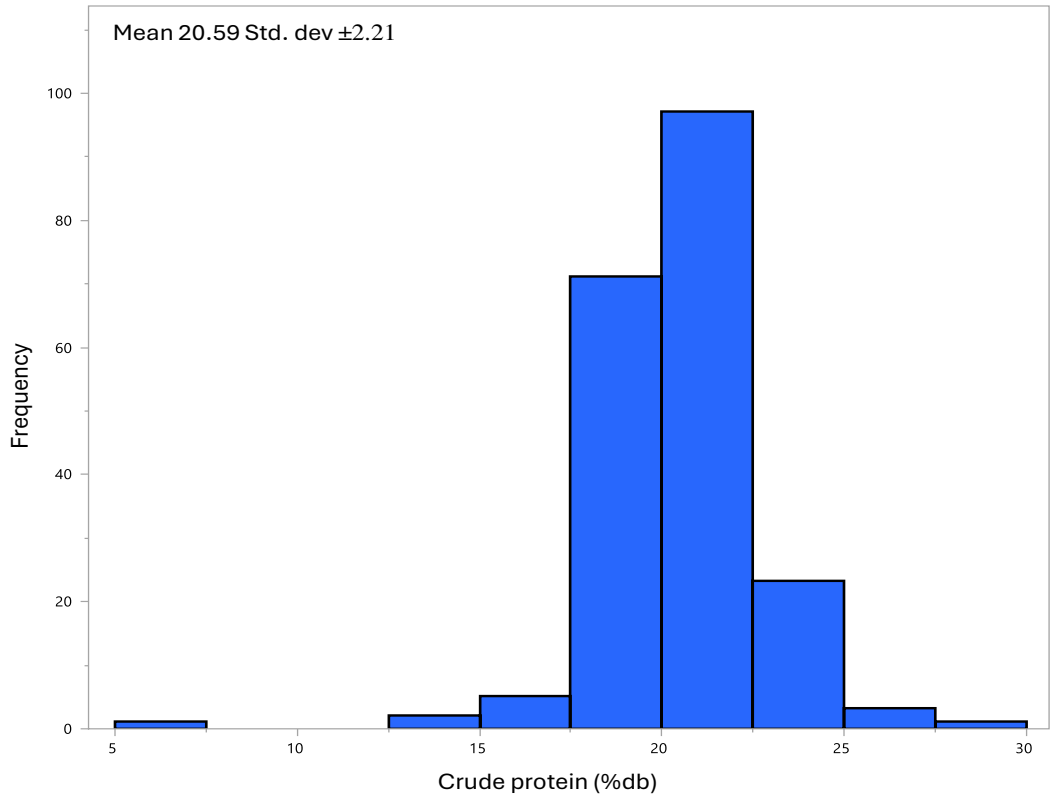

Figure 3.1 Frequency distribution of crude protein content in seeds with of Osimo_2019 trial.

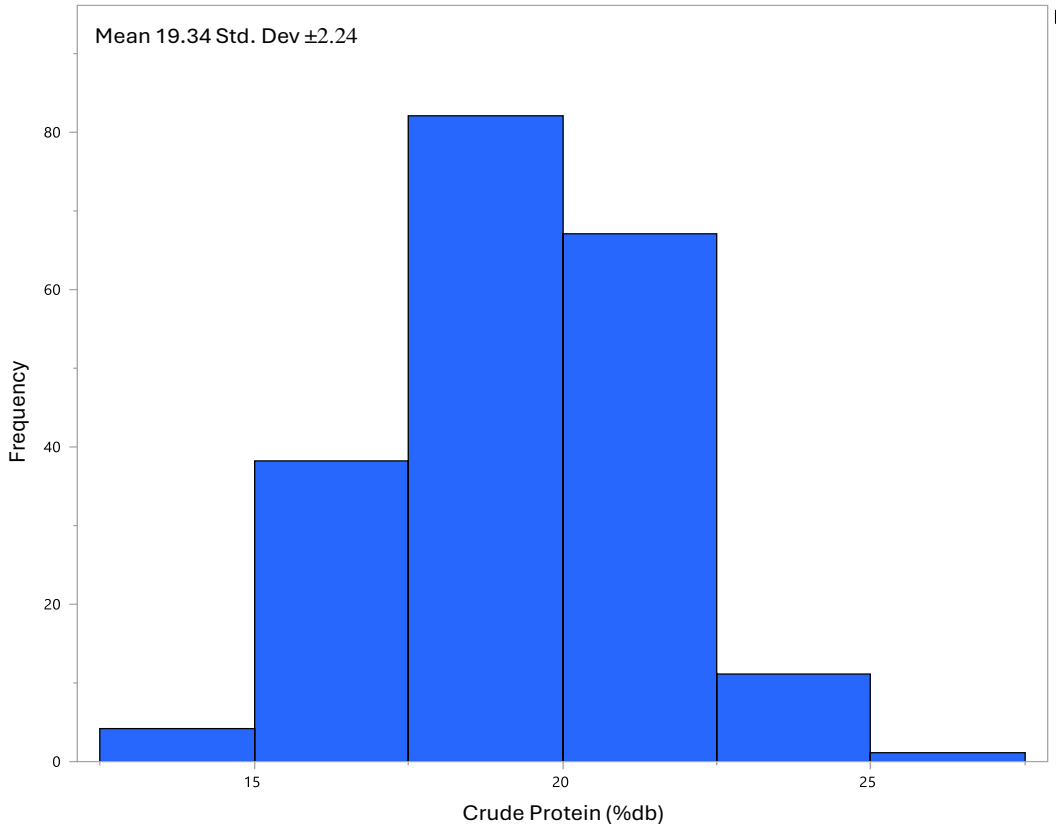

Figure 3.2 Frequency distribution of crude protein content in seeds of the Osimo_2021 trial.

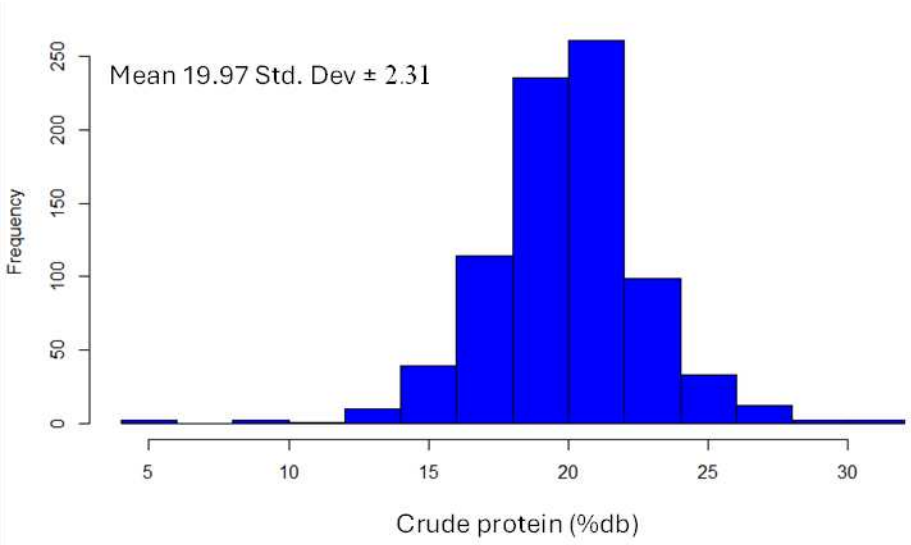

Figure 3.3 Frequency distribution of crude protein content in seeds of Osimo_2019 and Osimo_2021 trials.

3.1.2 DESCRIPTIVE STATISTICS FOR MARKET TYPES

Table 3.2 reports descriptive statistics for chickpeas grouped based on market types, the Desi and Kabuli. We considered data from both trials, Osimo_2019, Osimo_2021, and the combined dataset. In Osimo_2019, the Desi group had a mean crude protein content of 19.39% with a CV of 12.14%, while the Kabuli group had a comparable mean of 19.24% but a slightly lower CV of 10.44% (Table 3.2 and Figure 3.4).

In Osimo_2021, both groups exhibited increased mean protein levels compared to seeds harvested in the 2019 trial; in particular, the Desi group showed an average of 20.56% for protein content with CV of 9.9%, while the Kabuli group showed a mean protein content of 20.66% with a CV of 12.33% (Table 3.2 and Figure 3.5). Similar results were found for the combined data (Table 3.2 and Figure 3.6). These results indicate no significant difference was detected between the two market types for protein content. A t-test analysis was provided to prove that (Figure 3.7, 3.8, and 3.9).

Environments	Market groups	Mean	Std.Dev	Min	Max	Range	CV
Osimo_2019	Desi	19.39	±2.35	13.68	27.47	13.84	12.14
	Kabuli	19.24	±2.01	15.33	24.38	8.84	10.44
Osimo_2021	Desi	20.56	±2.03	12.72	26.69	13.97	9.9
	Kabuli	20.66	±2.55	7.49	28.66	28.66	12.33
Osimo2019/2021	Desi	19.98	±1.68	14.57	25.06	10.58	8.42
	Kabuli	19.95	±1.76	12.72	24.17	11.46	8.83

Table 3.2 Descriptive statistics for chickpea genotypes grouped by market types across various environments.

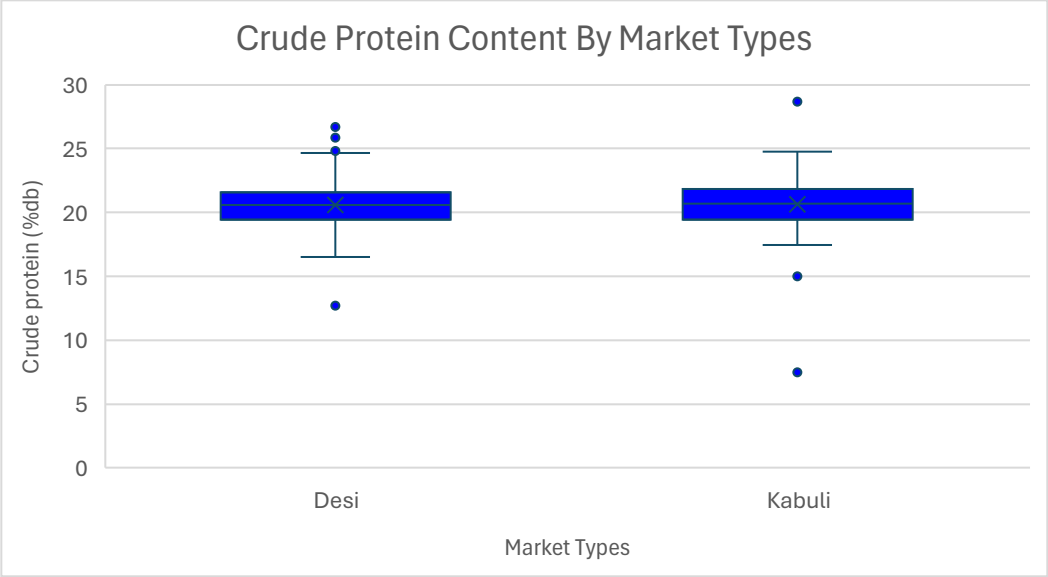

Figure 3.4 Boxplot for protein content of seeds of genotypes grouped based on market types (2019 trial)

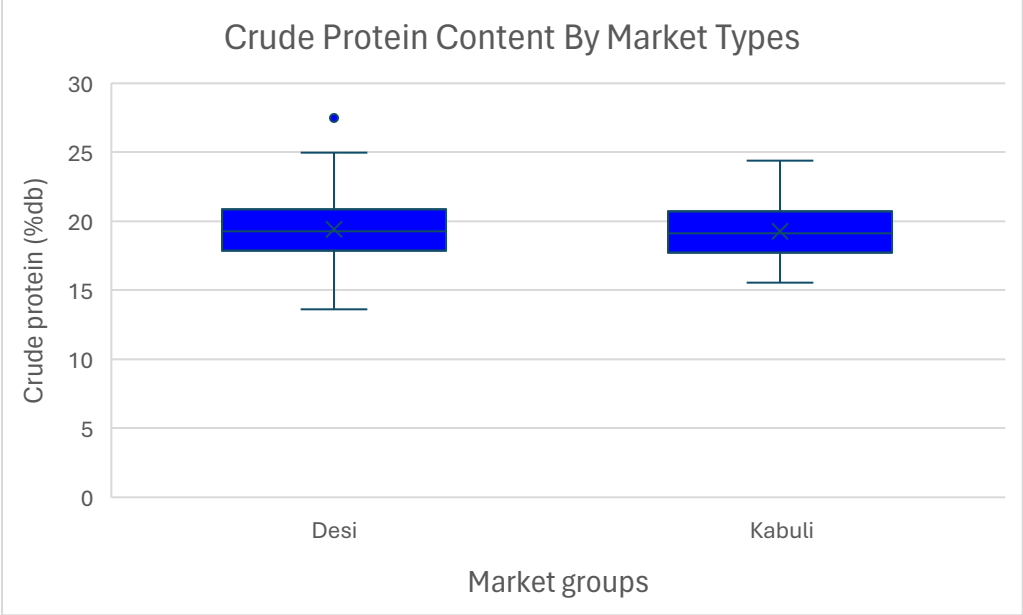

Figure 3.5 Boxplot for protein content of seeds of genotypes grouped based on market types (2021 trial)

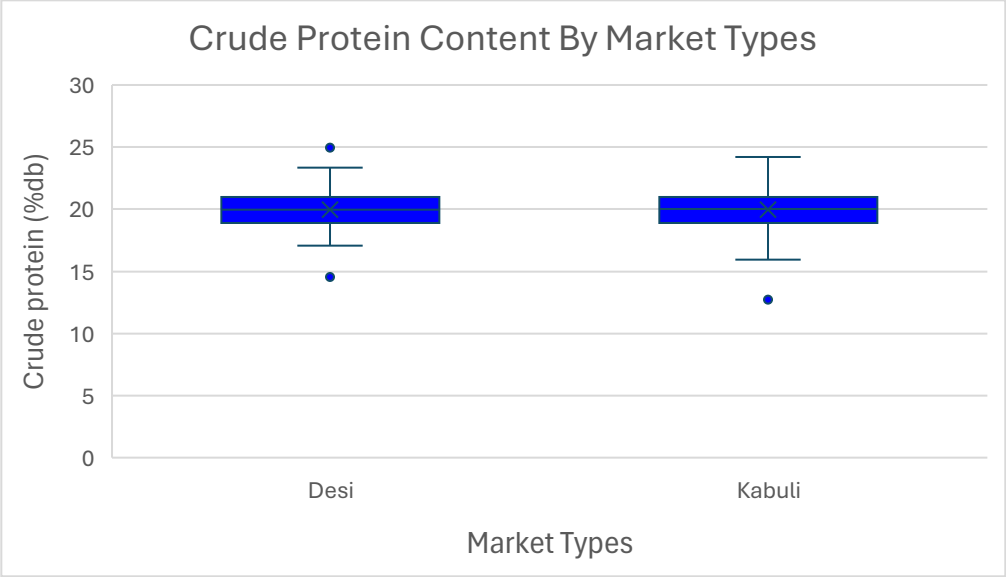

Figure 3.6 Boxplot for protein content of seeds of genotypes grouped based on market types (combined data)

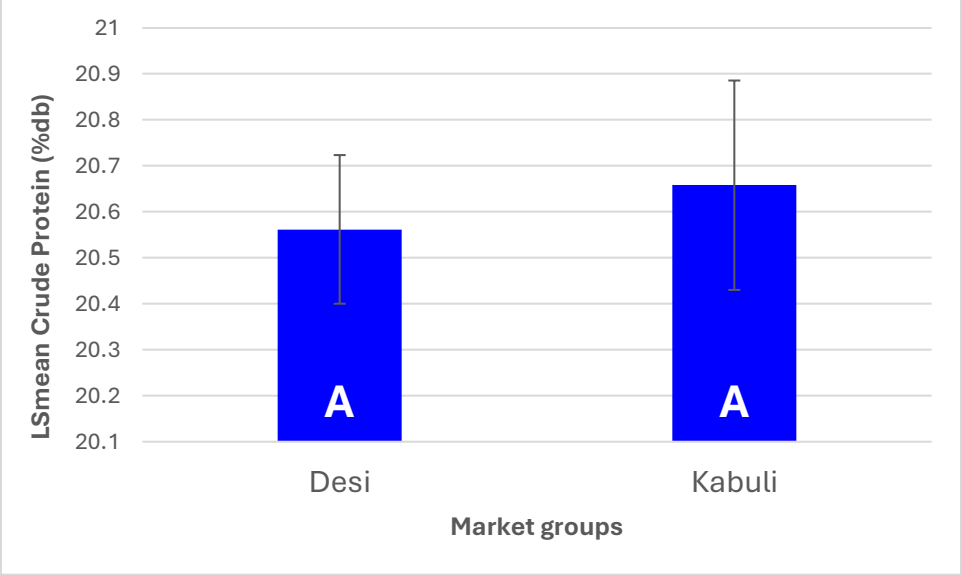

Figure 3.7 T-test analysis of crude protein content between the two market groups of chickpeas from the Osimo_2019 trial

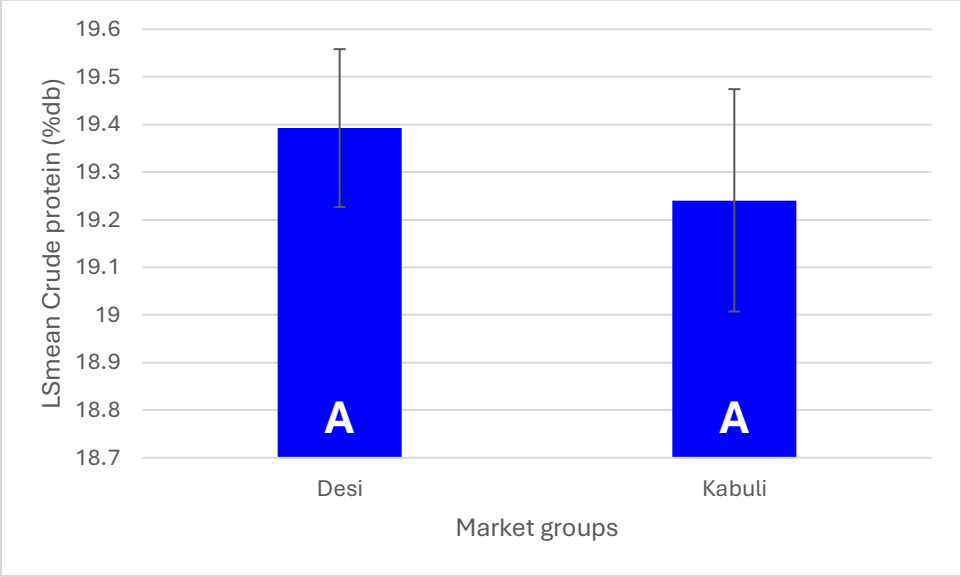

Figure 3.8 T-test analysis of crude protein content between the two market groups of chickpeas from the Osimo_2021 trial

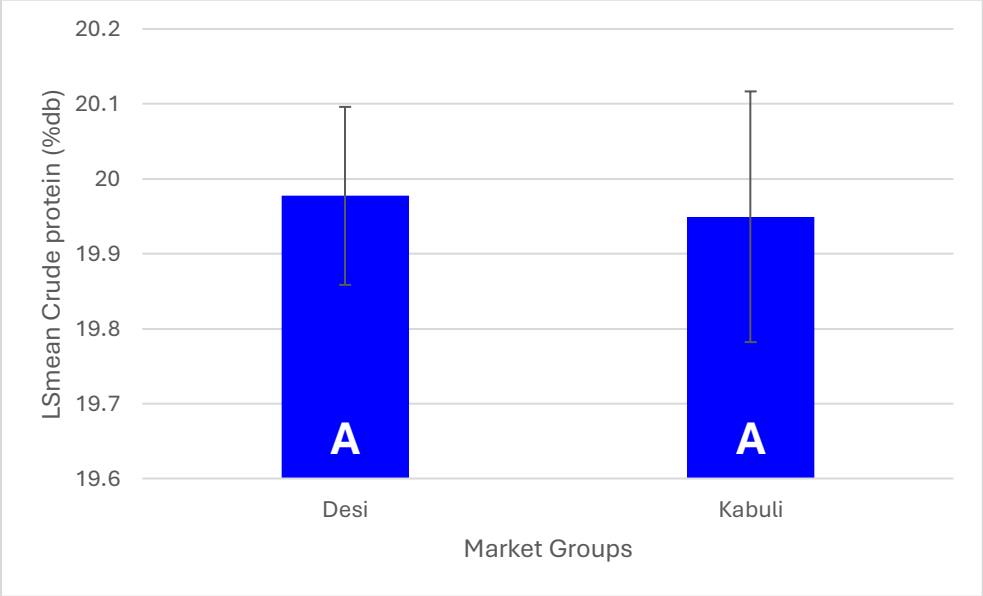

Figure 3.9 T-test analysis of crude protein content between the two market groups of chickpeas from the combined Osimo_2019 and 2021 trial

The crude protein content of seeds grown in Osimo_19 and Osimo_21 was similar (Figure 3.10). This indicates moderate variability in protein content in both years, with no substantial difference. Outliers are present in both environments, though Osimo_19 has more extreme values, with outliers ranging from about 5% to close to 30%. For Osimo_21 fewer extreme outliers were detected. For the box plot, the replicates were used separately.

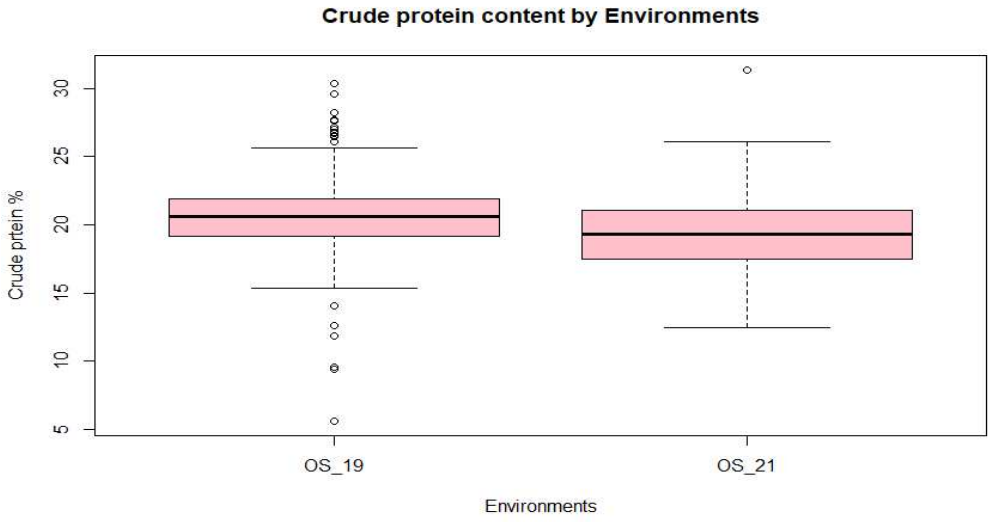

Figure 3.10 Boxplot for protein content of seeds of genotypes grouped based on market types (combined data).

3.2 RANDOM EFFECT ANALYSIS AND HERITABILITY AND GEI ANALYSIS

Tables 3.3, 3.4, and 3.5, are reported the broad-sense heritability (H^2) estimates for protein content considering data from 2019, 2021, and both trials. For the 2019 trial, we detected a significant genotype effect for protein content, with heritability equal to 39.01%. Residual variation contributed 60.99%, indicating that a significant portion of the variance is not explained, and the presence of other factors influences the protein content (Table 3.3). For 2021, heritability was slightly lower (35.67%) than that related to the 2019 field trial (Table 3.4). Considering data from both trials, we detected a significant contribution of GEI on protein content variation (23.17%), highlighting genotype-specific responses across environments, as well as a significant genotype effect (10.60%; Table 3.5; Figure 3.8). Residuals remained the main source of unexplained variation (56.66%).

Table 3.3 Random effect analysis and heritability for crude protein,2019 trial

Random Effect	Var Ratio	Var Component	Std Error	95% Lower	95% Upper	Wald p-Value	Pct of Total
Genotypes	0.64	2.75	0.53	1.71	3.79	<.0001	**39.01**
Reps	0.00	0.00	0.03	-0.06	0.06	0.99	0
Residual		4.30	0.43	3.57	5.28		60.99
Total		7.05	0.53	6.11	8.22		100

Table 3.4 Random effect analysis and heritability for crude protein, 2021 trial

Random Effect	Var Ratio	Var Component	Std Error	95% Lower	95% Upper	Wald p-Value	Pct of Total
Genotypes	0.56	2.64	0.55	1.56	3.73	<.0001	**35.67**
Reps	0.00	0.02	0.05	-0.09	0.12	0.78	0.207
Residual		4.75	0.47	3.95	5.84		64.12
Total		7.41	0.55	6.44	8.63		100

Table 3.5 Random effect analysis and heritability for crude protein across environments.

Random Effect	Var Component	Std Error	95% Lower	95% Upper	Wald p-Value	Pct of Total
Genotypes	0.85	0.36	0.15	1.55	0.0168	10.60
Environment	0.76	1.11	-1.41	2.93	0.5	9.47
Genotypes*Environment	1.86	0.44	0.99	2.72	<.0001	23.17
Reps [Environment]	0.01	0.03	-0.05	0.07	0.79	0.10
Residual	4.54	0.32	3.97	5.24		56.66
Total	8.01	1.17	6.13	10.92		100

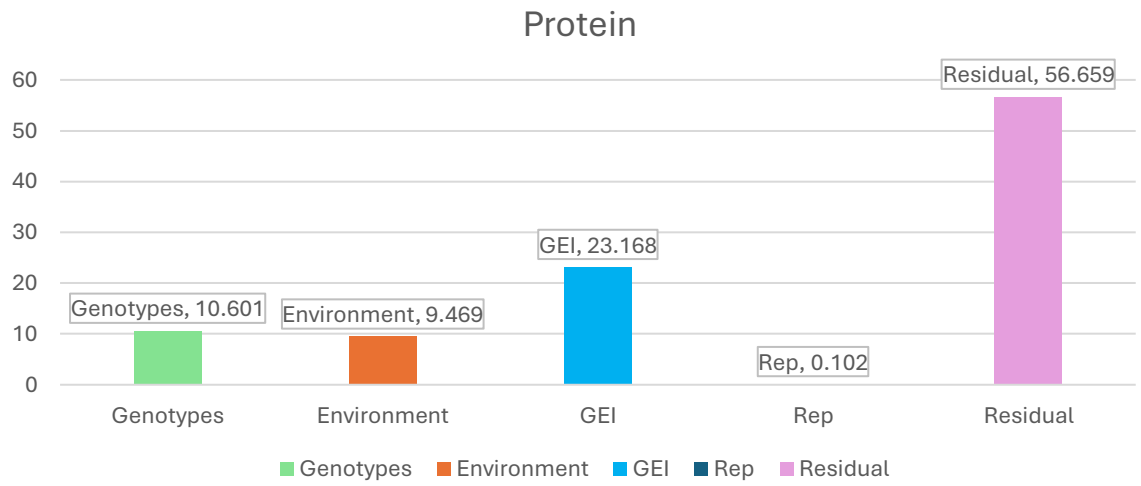

Figure 3.11 Genotype, environment, GEI, replicate, and residual contribution to protein content variation of chickpea seeds.

We then tested the statistical significance of genotype, environment, and GEI by treating them as fixed factors. Significant contributions of genotype, environment, and GEI were detected (Table 3.6).

Table 3.6 The analysis of variance (ANOVA) table for crude protein content

Source	DF	Sum of Squares	F Ratio	Prob > F
Genotypes	202	2347.1939	2.5621	<.0001
Environment	1	318.3683	70.1994	<.0001
GEI	202	1661.759	1.8139	<.0001

We detected a clear effect of the environment to determine crude protein content, with significantly lower protein content in seeds obtained in Osimo_21 compared to Osimo_19. This result suggests the importance of considering environmental factors for protein content. Figure 3.12

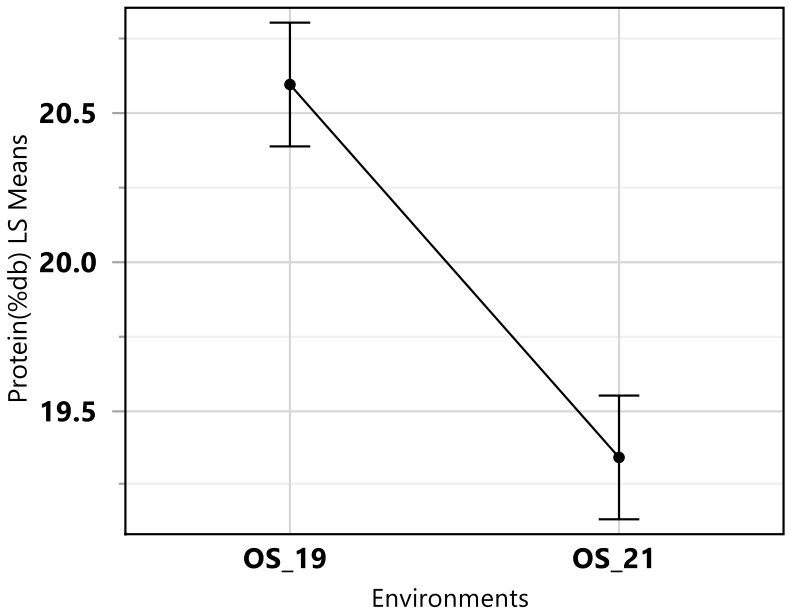

Figure 3.12 Least squares mean of crude protein content across environments (2019 vs 2021)

3.3 STABILITY EVALUATION

To investigate the stability and performance of different genotypes, we used the genotype-grouping technique as proposed by Francis and Kennenberg (1978). By this procedure, the protein content means of each genotype averaged over environments was plotted against its coefficient of variation (CV) over environments.

Figure 3.8 illustrates a biplot of mean protein content versus CV for the analyzed genotypes. The plot categorizes genotypes based on their performance and stability. Genotypes such as An_Ca_0110, An_Ca_0175, An_Ca_531, and An_Ca_0460 displayed high mean seed protein content with low CVs, indicating consistent performance with minimal variability across different environments. These genotypes were classified within Group I, signifying their potential as stable, high-protein performers suitable for broad adaptation. Conversely, genotypes including An_Ca_1343, An_Ca_887, An_Ca_0601, An_Ca_0129, and An_Ca_0580 consistently exhibited low mean seed protein content across both environments. These genotypes were placed in Groups III and IV, indicating low protein levels with varying degrees of stability. Group III included genotypes with low protein content coupled with high

variability, reflecting environmental sensitivity, while Group IV comprised genotypes with low protein content but low variability, suggesting stable yet suboptimal performance.

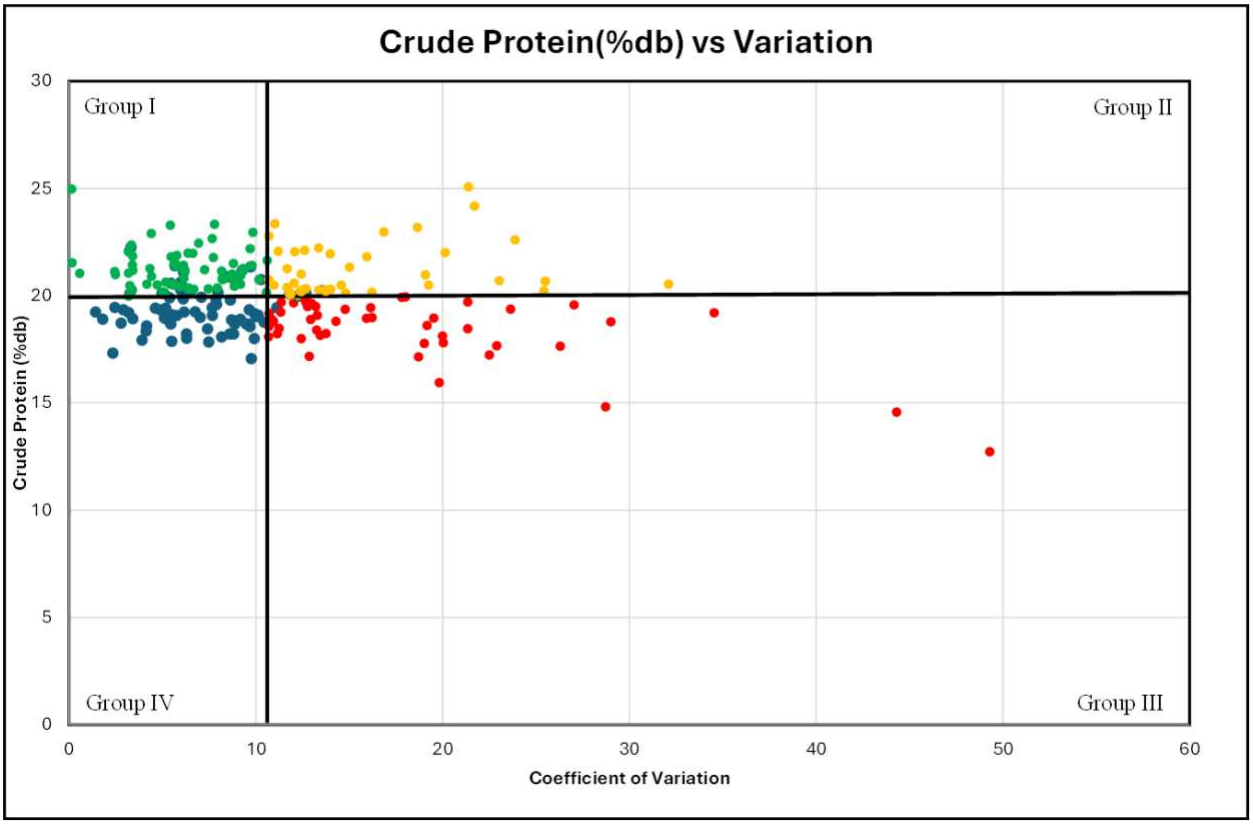

Figure 3.13 Mean protein content vs. CV. Grouping categories: Group I(green), high mean, low variability; Group II(Yellow), high mean, high variability; Group III(Red), low mean, high variability; Group IV(Blue), low mean, low variability.

4 DISCUSSIONS

In the present study, we aimed to investigate the phenotypic diversity for protein content of a wide collection of domesticated chickpea germplasm (202 different genotypes) and to improve our knowledge of the factors that influence seed protein content in chickpeas. Indeed, this knowledge is crucial for developing improved cultivars with higher nutritional quality. Seed protein content is a complex trait governed by both genetic and environmental factors, with genotype-by-environment interactions (GEI) playing a significant role in shaping its variability.

Our findings reveal substantial variability in protein content within the collection, with a range from 7.49% to 28.66%. We identified an average protein content of 19.97%, which agrees with the data present in the literature (Wang et al. 2017; Summo et al. 2019; Srungarapu et al. 2022; Sari et al. 2024). Environmental conditions, including temperature, precipitation, and soil characteristics, play a significant role in determining seed composition (Wang et al. 2017).

The trials carried out in the present study were characterized by a major difference in environmental conditions, that is the year 2021 was characterized by the absence of rain during the last two months (June and July) of the growing cycle, so can be considered as an experiment carried out in drought conditions. We detected less variation among genotypes in the Osimo_21 trial and lower protein content, giving further evidence that higher temperatures during critical growth stages can lead to reduced protein synthesis and increased carbohydrate accumulation, negatively impacting overall protein content (Gaur et al., 2017).

Heritability estimates were equal to 39.01% and 35.67% in the 2019 and 2021 trials, respectively. These low-intermediate estimates suggest that it is possible to improve the trait by breeding but also that a consistent effect of environmental factors and additional variables affect the level of protein content, as also highlighted in the literature (Wang et al. 2017).

GEI effect (23.17%) variation observed in chickpea seeds of our collection for protein content resulted significantly higher than that of genotype (10.60%) and environment (9.47%). This result indicates that the response of diverse chickpea genotypes to variation in environmental factors was different, implying that selection of environment-specific genotypes is required, and numerous multi-location field trials need to be carried out exploring a wide set of different agro-environmental conditions.

On the other hand, the genotype–grouping technique allowed the identification of genotypes (e.g. An_Ca_0110 and An_Ca_0175) that showed high protein content and stability for such traits in both the two environments, suggesting that they are more adaptable and can be used in a broad variety of agro-climatic conditions.

5 CONCLUSION

Chickpea is recognized for its nutritional value and adaptability to various environmental conditions, making it a vital crop for human and livestock nutrition globally. Here, we characterized for protein content a wide set of 202 chickpea genotypes encompassing the worldwide geographic distribution of cultivated chickpeas derived from the EMCAP (European and Mediterranean Chickpea Association Panel) developed by Rocchetti et al. (2020). We investigated the variability of crude protein content and the genotype, environment and GEI effects on its variation. We identified high variability within our set of materials that can be exploited in breeding programs aimed at improving the nutritional quality of chickpeas. Heritability estimates, even if at a low-intermediate level, indicate that selection could be effective in improving the nutritional quality of chickpea varieties. Moreover, we also identified interesting materials that showed stable protein content across the two field trials that can be utilized in breeding programs to develop broadly adapted and of high nutritional quality varieties.

Finally, the significant and high effect of GEI also indicates the importance of conducting more field trials in different locations and environments to identify genotypes adapted to specific environments (high-performing genotypes with environment-specific adaptability).

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REFERENCES

Aguilera, J. M., Park, D. J., & Rojas-Graü, M. A. (2009). Food powder engineering: Characteristics, processes and applications. *Trends in Food Science & Technology*, 20(10), 493-507.

Ahmad, F., Gaur, P. M., & Slinkard, A. E. (1992). Chickpea breeding for resistance to abiotic and biotic stresses. *International Chickpea Newsletter*, 26, 10-14.

Ahmad, F., Slinkard, A. E., & Malhotra, R. S. (2005). Germplasm resources of chickpea. In *Plant Genetic Resources: Their Conservation and Utilization*. CAB International.

Alajaji, S. A., & El-Adawy, T. A. (2006). Nutritional composition of chickpea (Cicer arietinum L.) as affected by microwave cooking and other traditional cooking methods. *Journal of Food Composition and Analysis*, 19(7), 806-812.

Angulo-Bejarano, P. I., Verdugo-Montoya, N. M., & Osuna-Castro, J. A. (2008). Effect of fermentation on protein digestibility in chickpeas. *Journal of Agricultural and Food Chemistry*, 56(1), 12-18.

Beres, B. L., et al. (2010). Performance stability of Canadian wheat cultivars in variable growing environments. *Canadian Journal of Plant Science*, 90(1), 59-67.

Bhaumik, S., et al. (2018). Temperature resilience and drought tolerance in chickpea genotypes. *Field Crops Research*, 228, 107-115.

Boye, J., Zare, F., & Pletch, A. (2010). Pulse proteins: Processing, characterization, functional properties and applications in food and feed. *Food Research International*, 43(2), 414-431.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1), 248-254.

Chitra, U., et al. (1995). Protein digestibility in vitro in various Indian pulses. *Journal of Food Science and Technology*, 32, 432-435.

Clemente, A., Vioque, J., & Sánchez-Vioque, R. (1998). Effect of different heating and soaking processes on protein solubility and amino acid composition of chickpea. *Food Chemistry*, 62(3), 275-283.

Crossa, J., et al. (2017). The use of multi-environment trials to assess genotype performance across diverse conditions. *Theoretical and Applied Genetics*, 130(3), 639-659.

Dhawan, K., et al. (1991). Nutritional and chemical composition of chickpea genotypes. *Journal of Agricultural and Food Chemistry*, 39, 1455-1461.

Doyle, J. J., & Luckow, M. A. (2003). The rest of the iceberg. *Legumes (Fabaceae) in the context of plant phylogeny*. *Plant Physiology*, 131(3), 900-910.

Duke, J. A. (1981). Handbook of legumes of world economic importance. *Springer*.

FAO. (2022). Annual worldwide chickpea yields and production trends. *Food and Agriculture Organization Statistical Database*.

FAOSTAT. (2022). World legume production statistics. Retrieved from http://www.fao.org/faostat.

Francis, T. R., & Kannenberg, L. W. (1978). Yield stability studies in short-season maize: A descriptive method for grouping genotypes. *Canadian Journal of Plant Science*, 58(4), 1029-1034.

Frimpong, E. A., et al. (2009). Evaluation of CHN content in chickpea germplasm for nutritional breeding. *Agricultural Research Journal*, 23(1), 35-47.

Gaur, P. M., et al. (2017). Breeding for higher seed protein content in chickpea. *Crop Science*, 57(1), 54-61.

Gaur et al. (2017): "Baseline status and effect of genotype, environment, and genotype x environment interactions on iron and zinc content in chickpeas." *Euphytica*.

Getachew, A. (2019). Pulses and smallholder farmers in sub-Saharan Africa. *Development and Agronomy Journal*, 12(3), 75-88.

Graham, P. H., & Vance, C. P. (2003). Legumes: Importance and constraints to greater use. *Plant Physiology*, 131(3), 872-877.

Guillon, F., & Champ, M. (2002). Carbohydrate fractions of legumes: Uses in human and animal nutrition. *Trends in Food Science & Technology*, 13(1), 1-12.

Gupta, Y. P., & Dhillon, B. S. (1993). Protein and lipid composition of chickpea seeds. *Legume Research*, 16(1), 7-12.

Hasanuzzaman, M., et al. (2019). Legume utilization for sustainable agriculture. *Frontiers in Plant Science*, 10, 673.

Haug, W., & Lantzsch, H. J. (1983). Sensitive method for the rapid determination of phytate in cereals and cereal products. *Journal of the Science of Food and Agriculture*, 34(12), 1423-1426.

Helbaek, H. (1970). The beginning of food production in the Old World. *American Antiquity*, 35(1), 79-90.

Heslot, N., et al. (2012). Integration of environmental covariates in whole-genome prediction models for complex traits. *G3 Genes, Genomes, Genetics*, 2(6), 707-720.

Hirst, K. K. (2019). Origins and spread of agriculture in the Fertile Crescent. Retrieved from ThoughtCo website.

Hosseinpour-Niazi, S., et al. (2015). Legumes and type 2 diabetes: Systematic review and meta-analysis. *The American Journal of Clinical Nutrition*, 102(2), 412-424.

Huang, X., et al. (2015). Genome-wide association studies of 14 agronomic traits in rice. *Nature Genetics*, 47(5), 645-652.

Iruela, M., et al. (2002). Molecular markers associated with resistance to Ascochyta blight in chickpea. *Theoretical and Applied Genetics*, 104(4), 1026-1032.

Jain, M., et al. (2017). Whole genome sequencing of chickpea provides a resource for trait improvement. *Nature Biotechnology*, 35(10), 926-932.

Jambunathan, R., & Singh, U. (1981). Studies on desi and kabuli chickpea cultivars. *Journal of Food Science*, 46, 1364-1367.

Jiang, Y., & Zhang, T. (2019). Genomic selection and marker-assisted selection in chickpea breeding. *Theoretical and Applied Genetics*, 132(8), 2349-2360.

Jones, D. B. (1931). Factors for converting percentages of nitrogen in foods and feeds into percentages of protein. *United States Department of Agriculture Circular*, 183.

Jukanti, A. K., et al. (2012). Nutritional quality and health benefits of chickpea. *The Scientific World Journal*, 2012, 12 pages.

Kaiser, W. J., et al. (1994). Biology and pathogenicity of Ascochyta rabiei on chickpea. *Plant Disease*, 78(10), 917-921.

Kazan, K., & Muehlbauer, F. J. (1991). Toxin production by Ascochyta rabiei. *Mycopathologia*, 114(1), 17-22.

Khan, A. et al. (2017). Drought tolerance mechanisms in chickpea: Advances in physiology, breeding, and molecular genetics. *Plant Breeding*, 138(2), 145-161.

Khan, M. A., et al. (2019). Drought tolerance mechanisms in chickpea: Advances in physiology, breeding, and molecular genetics. *Plant Breeding*, 138(2), 145-161.

Kumar et al. (2020): "Baseline status and effect of genotype, environment, and GEI on iron and zinc content in Indian chickpeas." *Euphytica*.

Ladizinsky, G., & Adler, A. (1976). The origin of chickpea: Cicer arietinum L. *Economic Botany*, 30(4), 257-264.

Lewis, G. P., Schrire, B., Mackinder, B., & Lock, M. (2005). Legumes of the world. *Royal Botanic Gardens, Kew*.

Lowry, O. H., et al. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193(1), 265-275.

Mackay, I., et al. (2009). Relevance of genome-wide association studies in complex trait breeding. *Theoretical and Applied Genetics*, 118(7), 1103-1120.

Maheri-Sis, N., et al. (2008). Nutritional composition of chickpeas and related legume species. *Journal of Food Science*, 73(2), 27-33.

Manickavelu, A., et al. (2019). Advances in chickpea breeding: New techniques and challenges. *Breeding Science*, 69(1), 56-68.

Mandal, S., et al. (2021). Identification of QTLs for drought tolerance in chickpea through GWAS. *BMC Plant Biology*, 21(1), 1-12.

Marzo, F., et al. (2002). Effects of extrusion on nutritional properties of legumes. *Journal of Agricultural and Food Chemistry*, 50(1), 403-409.

May, W. E., et al. (2010). Impact of genotype, environment, and genotype-by-environment interactions on yield and yield stability of oat. *Crop Science*, 50(4), 1360-1369.

Mondor, M., et al. (2009). Influence of processing on the protein quality and content of chickpeas. *Food Chemistry*, 113(4), 1102-1108.

Moumeni, A., et al. (2016). Chickpea growth, physiology, and productivity under different stress conditions. *Environmental and Experimental Botany*, 130, 11-20.

Muoni, T., et al. (2019). Nutritional and environmental benefits of legumes in mixed farming systems. *Frontiers in Plant Science*, 10, 770.

Muehlbauer, F. J., et al. (1994). Taxonomy and germplasm resources of chickpea (Cicer arietinum L.). *In Genetic Resources and Their Exploitation*, 65-85.

Nedumaran, S., et al. (2015). Grain legume production and productivity in developing countries. *Agricultural Research*, 4(3), 194-204.

Patterson, H. D., & Thompson, R. (1971). Recovery of inter-block information when block sizes are unequal. *Biometrika*, 58(3), 545-554

Peumans, W. J., & Van Damme, E. J. M. (1996). Lectins as plant defense proteins. *Plant Physiology*, 109(1), 347-352.

Prakash, J., & Prakash, U. (1999). Nutritional value and digestibility of chickpeas in relation to other legumes. *Plant Foods for Human Nutrition*, 54(1), 47-60.

Rasool, A., et al. (2015). Comparative analysis of nutrient composition in desi and kabuli chickpea. *Legume Research*, 38(4), 456-462.

Roy, F., et al. (2010). Factors influencing protein content and quality in chickpeas. *Journal of the Science of Food and Agriculture*, 90(4), 543-548.

Sames, T., et al. (2001). Potential therapeutic applications of lectins in obesity and cancer prevention. *Trends in Pharmacological Sciences*, 22(4), 172-180.

Sandhu, N., et al. (2021). Predicting yield stability of chickpea using genomic selection models. *Frontiers in Plant Science*, 12, 231.

Sari, H., Uhdre, R., Wallace, L. *et al.* Genome-wide association study in Chickpea (*Cicer arietinum* L.) for yield and nutritional components. *Euphytica* **220**, 84 (2024). https://doi.org/10.1007/s10681-024-03338-x

Segev, A., et al. (2010). Total phenolic content and antioxidant activity in chickpea sprouts and their seed coats. *Journal of Food Biochemistry*, 34(1), 56-65.

Singh, M. (1988). Antinutritional factors in chickpea and effects of processing methods. *Journal of Agricultural and Food Chemistry*, 36(4), 725-730.

Shah, T., et al. (2018). Effect of soil fertility and temperature on chickpea yield and GEI. *Environmental and Experimental Botany*, 155, 24-35.

Smith, P. K., et al. (1985). Measurement of protein using bicinchoninic acid. *Analytical Biochemistry*, 150(1), 76-85.

Srungarapu, R., Mohammad, L. A., Mahendrakar, M. D., Chand, U., Venkata, R. J., Kondamudi, K. P., ... & Samineni, S. (2022). Genetic variation for grain protein, Fe and Zn content traits in chickpea reference set. *Journal of Food Composition and Analysis*, *114*, 104774.

Summo, C., De Angelis, D., Ricciardi, L., Caponio, F., Lotti, C., Pavan, S., & Pasqualone, A. (2019). Nutritional, physico-chemical and functional characterization of a global chickpea collection. *Journal of Food Composition and Analysis*, *84*, 103306.

Thavarajah, D., et al. (2009). Phytic acid and mineral profile in chickpea cultivars. *Plant Foods for Human Nutrition*, 64(1), 74-82.

Tiwari, B. K., et al. (2009). Phenolic acids in chickpeas: Extraction, purification, and quantification. *Food Chemistry*, 116(3), 819-825.

Van der Maesen, L. J. G. (1987). Taxonomy of chickpea: An overview. *International Chickpea Newsletter*, 17, 5-8.

Xu, B., & Chang, S. K. (2009). Antioxidant activity and phenolic compounds of chickpeas. *Food Chemistry*, 113(2), 304-313.

Yadav, S. S., et al. (2021). Marker-assisted breeding for yield and protein content in chickpeas. *Field Crops Research*, 268, 108-117.

Zia-Ul-Haq, M., et al. (2007). Nutritional composition of desi chickpea and kabuli chickpea. *Pakistan Journal of Botany*, 39(5), 1695-1702.

Zhao, J., et al. (2009). Isoflavones in chickpeas and their bioactivity. *Journal of Agricultural and Food Chemistry*, 57(5), 2201-2207.

Wang, R., Gangola, M. P., Jaiswal, S., Gaur, P. M., Båga, M., & Chibbar, R. N. (2017). Genotype, environment and their interaction influence seed quality traits in chickpea (Cicer arietinum L.). *Journal of Food Composition and Analysis*, *63*, 21-27.