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SEED HETEROMORPHISM AND GERMINATION IN *CHENOPODIUM QUINOA* WILLD. RELATED TO CROP INTRODUCTION IN MARGINALIZED ENVIRONMENTS

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Abstract

Abiotic stress tolerance and the superior nutritional seed value make *Chenopodium quinoa* (Amaranthaceae) one of the most important candidates for crop diversification and food use. This article evaluates seed germination behavior, grain yield, pericarp, and seed coat structure of different seed heteromorphs in various quinoa lines, newly introduced on alkaline soils in Caspian lowlands. Introduction into harsh environments induces changes in expression, resulting in increased fruit and seed heterogeneity, expressed as variation in sizes and colors (light and dark), seed coat, and pericarp structure. These changes affect the seed germination, grain yield and other agronomic parameters. Light seeds predominanted, while the proportion of dark seeds varied from 9 to 17 percent in the quinoa lines examined. Tannins, lignin, and stalactites were detected in the cell walls of the exotesta of phenotypes of quinoa seeds. Early-maturing lines had a lower percentage of dark seeds, high germination rates in the laboratory, and synchronized seedling emergence in the field, followed by fast plant growth, high grain yield, and 1000-kernel weight. Caspian drylands are potential areas for the cultivation of early maturing quinoa genotypes, whose seed structural and functional features are not affected by stress conditions. Seed heteromorphism might represent an expectant seed survival strategy under changing environments.

Key words: *Chenopodium quinoa,* Drylands, Pericarp, Seed characteristics, Anatomy, Metabolites, Caspian region.

Introduction

The genus *Chenopodium* (150–200 species) is one of the largest and most polymorphic in the Amaranthaceae family (Fuentes-Bazan *et al*., 2012; Sukhorukov, 2014; Sukhorukov *et al*., 2018). The majority of *Chenopodium* species have black seeds with a thick, dark brown seed coat (Sukhorukov & Zhang, 2013; Sukhorukov, 2014). The seeds of *C. quinoa*, *C. pamiricum* Iljin, and *C. pallidicaule* Aellen are predominantly light colored, with thin yellow seed coats (Sukhorukov & Zhang, 2013; Sukhorukov, 2014; Abdelbar, 2018). In *C. album* and *C. pamiricum*, yellow or brownish seeds occur equally with black seeds. *C. album* is the most studied species in this respect; different authors distinguish two to four types of seeds. For instance, P. Levina (1987) highlighted three types: (1) large (up to 2 mm in diameter), flat, light brown; (2) smaller, less flattened, black; (3) very small, almost spherical, black. The data on seed heteromorphism in *C. album* are contradictory: (i) some authors describe dimorphic black and brown seeds with different seed coat thicknesses (Baar, 1913; Basset, Crompton, 1978; Sukhorukov & Zhang, 2013; Sukhorukov, 2014); (ii) others (Iljin & Vasilchenko,1934; Dobrokhotov, 1961; Levina, 1987) report seeds of three types, among which two types of black seeds differ morphologically and in germination rate (Dobrokhotov, 1961); (iii) there is evidence in the literature,where 4 seed morphs are distinguished-black or brown, both show reticulate or smooth seed coat surface (Williams & Hurper, 1965; Hurper, 1977; Matilla *et al*., 2005); and (iv) occasionally all seeds are considered to be the same (Iljin, 1936). Such discrepancies in the manifestation of seed heteromorphism phenomenon may be related to the impact from environmental stresses in particular soil salinity. Different populations of *C. album* also differ in the degree of seed heteromorphism. In *C. album*, morphologically similar black seeds are capable of either rapid or delayed germination, which is associated with different thicknesses of the seed coat; the thickness depends on day length: a thick seed coat is formed in summer and a thin one is formed in autumn (Sukhorukov, 2014). Seed color differences sometimes arise under unfavorable environmental conditions (Yao *et al*., 2010).

Response factors to salinity stress and accumulation of storage compounds in different seed structures have been intensively studied in many quinoa accessions (Jessica *et al*., 2020; Toderich *et al*., 2020). An X-ray microanalysis has revealed that Na^+ accumulation is high in the pericarp of quinoa seeds and low in the perisperm and embryo tissue (Sayed *et al*., 2017).

We have reported that excess soil salinity induces differences in growth among quinoa lines and affects the chemical composition and nutritive properties of seeds at the maturation stage (Choukr-Allah *et al*., 2016; Toderich *et al*., 2020). As highlighted by Prego *et al*., (1998) and Gasimova *et al.*, (2019), carbohydrate reserves in Quinoa seeds are localized in the large perisperm, which occupies the large central part of the mature seed (Fig. 8A). Externally, it is adjacent to the seed coat and the circular embryo, whose hypocotyl-radical axis is surrounded by one- or two-cell-layered endosperm (Fig. 8A-D), rich in proteins and oils like the embryo. Various chemical storage reserves were detected in the embryo, endosperm, and in the seed coat. Some chemical compounds of quinoa seeds, such as tannins and trypsin, which are localized mostly in the seed coat, inhibit seed germination (Prego *et al*., 1998;

Gasimova *et al*., 2019; El Hazzam *et al*., 2020). The use of quinoa grains is compromised by anti-nutritional secondary metabolites deposited in the seed coat (El Hazzam *et al*., 2020). The structural traits of fruits and seeds of *C. quinoa* have widely been studied to understand the role of metabolites (Varriano-Marston & De Francisco, 1984; Prego *et al*., 1998; Bojňansky & Fargašova, 2007; Van Raamsdonk *et al*., 2010; Sukhorukov & Zhang, 2013; Gomaa, 2014; Sukhorukov, 2014; Burrieza *et al*., 2014; Abdelbar, 2018; Gasimova *et al*., 2019; Gasimova, 2020).

There are evidences that new harsh environments affect seed dormancy and seed germination rate in *Chenopodium* species through changes in seed coat structural and chemical characteristics (Pourrat & Jacques, 1975; Ceccato *et al*., 2015). In *C. polyspermum* and *C. album*, seed coat thickness and germination depend on the photoperiod during seed development (Karssen, 1970). In *C. album*, seeds with darker seed coats have higher dormancy and their occurrence has been correlated with longer days (Karssen, 1970). Variations in seed dormancy level in *C.bonus-henricus* are associated with the altitude of the origin of this species (Dorne, 1981). According to McGinty *et al*., (2021) weak seed dormancy in Quinoa reduces yields because of premature germination before harvest; in fact the regulation of seed dormancy and germination process in quinoa is poorly understood.

In quinoa, seed dormancy, which is manifested in delay or absence of seed germination, occurs after seed maturation and could be due to the environmental impacts, rather than inherited (McGinty *et al*., 2021). We think that fruit and seed coat diversity in quinoa seed heteromorphs are determined by harsh growth conditions and to a lesser extent by seed structural and functional features.

Our aim here was to investigate the germination rate and morphology of heteromorphic fruits and seeds in four quinoa genotypes (improved lines) newly introduced to saline soils of Caspian lowlands. The specific goals were: (1) to characterize the proportions and agronomic features of seed morphs; (2) to reveal structural differences among heteromorphic fruits and seeds (especially pericarp and seed coat features) and metabolite profiling in mature seeds; and (3) to examine the variability of seed morphology related to germination, crop performance, and grain yield within and among the quinoa lines introduced to Caspian drylands.

Materials and Methods

Characteristics of research target area: The Kur-Araz lowland, with an area of about 2.2 million hectares, occupies a central part in Azerbaijan separating the Greater and Lesser Caucasus and washed by the Caspian Sea in the east (Fig. 1).

Fig. 1. Map of degraded salt affected lands in Kur-Aras (Azerbaijan), where Kurdamir Experimental Station (KES) is located. Quinoa field trials are marked in blue (Anon., 2014).

The Kur-Araz lowland is a typical arid zone characterized by a dry climate with a prolonged hot summer and short, relatively mild, winters. The average temperature is 25°C-28°C in July and 1.3°C-3.6°C in January, the precipitation is 200-380 mm; surface evaporation being 3.0-3.5 fold the amount of atmospheric precipitation. The region is the largest irrigated agricultural territory in Azerbaijan and faces multiple ecological problems, including accumulation of surplus of salts and secondary salinization as a result of inappropriate irrigation of agricultural lands. Intensive irrigation of agricultural lands is also elevating the groundwater table and consequently increasing the salt content of the crop root zone. Most of this area has been converted into marginal degraded lands through agricultural use, and the soils have become unfertile.

Field trial and soil characteristics: The experiments were carried out at the KES of the Institute of Botany, Azerbaijan National Academy of Sciences, during 2018-2020. KES is located in the central part of the Kura-Araz saline depression. The soil characteristics have been reported in Mamedov *et al*., (2020). Briefly, soil salinity is moderate (EC = 2.0-14 dS m^{-1}) in the upper layers (0-30 cm) but increases with depth. Ground water with mineralization of 6-15 g l^{-1} occurs 1.5-2.1 m from the soil surface. The soils have high bulk density (up to 1.43 g cm^{-1}), and clay soil shows crusting with a high swelling potential. Plants were grown in soil with a heavy clay texture and chloride-sulfate-type salinity ranging from moderate to high (EC = 6-12 dS m^{-1}) with a pH 8.0-9.1. The soil had low organic matter content (<12-15 ppm) and low texture.

Plant material: Salt-tolerant quinoa (*C. quinoa* Willd.) improved genetic lines Ames 13742 (primarily marked as ICBA-Q2), Ames 13761 (ICBA-Q3), Ames 22157 (ICBA-Q4) and NSL 106398 (ICBA-Q5) were obtained from the International Center for Biosaline Agriculture (ICBA), Dubai, UAE through a Material Transfer Agreement.

Design of field trials and crop traits: Original seeds of Q2-Q5 genotypes were for the first time planted at KES. The trial design, crop growth parameters including field observations on grain yield were carried out according to the guidelines developed by ICBA HQ (Nanduri *et al*., 2019). The field trials were laid out in a randomized complete block design (RCBD) with three replicates. The distance between rows was 60 cm, depth of planting was 1 cm. Two seeds of each quinoa accession were sown in a cell of the seed tray. After germination additional seedlings were removed and one plant was kept per cell. NPK Fertilizer, zero or 120-40-40 kg ha^{-1}) and microelements of 4-12 (g ha^{-1}) were applied. Furrow irrigation at 200-600 mm (total rain + irrigation water depth ~2000-4000 mm) was conducted approximately every 2-4 weeks in accordance with the soil water content, which was measured by using a soil moisture sensor (ES-5, Decagon Devices), and the rain pattern (Mamedov *et al*., 2020).

Agronomic data such as plant height, number of branches, inflorescence length and width seed yield and fresh and dry biomass yields were recorded on plants from a 1 m^2 quad rat demarcated from the middle of each plot excluding the border rows.

Phenological stages were recorded as active growth stage, full flowering days, physiological maturity, and grain maturity. Agronomic trait measurements were based on standard plant guidelines (Nanduri *et al*., 2019, Gasimova *et al*., 2018). The plant height was measured from the soil surface to the tip of the panicle, duration of crop cover was determined from seedling emergence to full maturity of seeds (Gasimova, 2020).

Flowering time was assessed in each genotype/quinoa improved line. The criteria to evaluate were; when the buds have started flowering was the exhibition of anthers in the opened flowers. Data was recorded on five dates at the end of May end of June. The number of days required to achieve the 90 % of plants with flowers was calculated from a simple linear regression equation. Seed harvest was done in September. The hardness of the grains when pressed against the thumb's fingernails was used as a criterion to determine the appropriate harvest date. The number of broken plants in the field was recorded to further correct mean biomass and grain yield values.

The grain yield was calculated per one plant. The dry panicles/plant were crushed and sieved to separate the seeds from the rest of the heads. Another step for seed cleaning was the removal of remaining parts (including dust) with an air blowing machine. Dry weight of remains (stem, leaves, dust) and clean seeds were documented. With available data of stems dry weight was measured at 35^o and 105^oC, the percentage of dry matter was calculated using the formula:

$$DM\ \% = (DW\ 105^oC\ in\ g / DW\ 35^oC\ in\ g) \times 100$$

These results were used to estimate the DW of seeds at 105°C with the formula:

$$DW\ 105^oC = DW\ 35^oC\ in\ g \times DM\ \%$$

The dry weights at 105°C for stem and the heads (without dust and seeds) were added as plant biomass. These results together with seed DW were extrapolated from the area of each plot (g/m^{-2}) to hectares with the formula:

$$DW\ (kg\ ha^{-1}) = (10 \times DW\ 105^oC\ (g) / m^{-2})$$

Seeds were hand harvested at physiological maturity at 20-30% water content (Gasimova *et al*., 2018, 2019). Fresh harvested fruits and seeds from KSE trials were used to examine morphotype heterogeneity (Gasimova, 2020).

The following morphological and physiological traits were recorded: plant height at maturity stage, the number of days to maturity, number of panicles per plant, panicle length, panicle width, grain yield, and 1000-seed weight after harvesting.

The thousand seed weight (TSW) of each Quinoa line was estimated. Samples consisted of three sets of 100 seeds randomly selected. The number of seeds was determined by an automatic seed counter and the weights were measured with an analytical balance. This variable was calculated as $TSW = (Weight\ in\ g/number\ of\ seeds) \times 1000$.

Seed germination testing in the laboratory and morphological measurements: Freshly harvested seeds from the first reproduction at KES were used; damaged and deformed seeds were discarded. Petri dishes were cleaned, rinsed with distilled water, and autoclaved at 120°C. Seeds (100 each in four replications) were placed in Petri dishes on filter paper soaked with 3 ml of distilled water. The Petri dishes were sealed with Parafilm to prevent water evaporation and kept in a growth chamber at a relative humidity of 70% and under constant light at 24°C (Khaitov *et al*., 2020). Seeds were considered germinated at a radicle length of at least 2 mm. Germinated seeds were counted every 24 h for up to 20 days.

The percentage of heteromorphic seeds was determined by counting 100 random samples in three replications. Seeds were scanned by using an Epson scanner and their diameter measured with the ImageJ (Medina *et al*., 2010) and GrainScan (Whan *et al*., 2014) programs, and the data were statistically analyzed in Excel 2016.

Light, Scanning Electron and Transmission Electron Microscopies analysis of seeds and fruits: For light microscopy, the material of the Q2 line was partly used fresh. Longitudinal median sections (12 or 24 µm thick) were taken on a freezing microtome and placed in glycerol. The sections were stained with phloroglucinol together with sulfuric acid, and with gentian-violet for lignin and with Sudan IV for cutin (Prozina, 1966). Tannins were identified by the characteristic brownish color of the tannin-impregnated cell walls (Danilova & Kirpichnikov, 1985).

Seed coat thickness was measured on longitudinal sections placed in glycerol on lateral seed sides; for each line, five light seeds with a diameter of 2.5-3.5 mm (large) and five seeds with a diameter of 1.5-2.0 mm (small) were measured (10 measurements per seed).

For scanning electron microscopy, a Stemi 2000-C stereomicroscope (Zeiss) and a Jeol JSM-6390 LA (Jeol) scanning electron microscope were used to study the micromorphology and internal structure of Q2-Q5 fruit and seed sections. As an illustrative material, a sole successfully dissected quinoa fruit from Dubai was used (Nanduri *et al*., 2019).

To prepare semi-thin sections (2-3 µm) fruits were fixed in 3% glutaraldehyde and 2% osmium tetroxide, dehydrated in a series of increasing concentrations of acetone in water (30-100%), embedded in a mixture of Epon and Araldite, and cut on an Ultracut-E ultramicrotome (Reichert-Yung). Sections were stained with 0.5% toluidine blue in a borate buffer. An AxioScope A1 (Zeiss) light microscope equipped with an AxioCam MRc5 digital video camera with Zen 2011 software was used to study the pericarp, seed coat, and storage tissue structure.

The seed surface classification and terminology suggested by Barthlott (1981), Murley (1951), and Stern (2004) were used to describe the surface structure.

Statistics: CropStat program was used for analysis of variance). Means and their standard errors were calculated. Differences were considered significant at $p<0.05$ (Tukey's test). Because differences among the various parameters in 2019 and 2020 were insignificant, the values were pooled and reanalyzed.

Results and Discussion

Seed germination in the laboratory and seedling emergence in the field: Wide variability was detected among the accessions (Figs. 2 and 3). Seed germination in the laboratory (average 71%; Fig. 2A) and seedling emergence in the field (93%; Fig. 2B) were high and uniform in Q5 and low in Q2 (<15%, Fig. 2A, B). Q4 had a prolonged seed germination period (Fig. 2C).

Agronomic performance of quinoa lines in field trials: Agronomic performance was examined in accessions with varied seedling emergence and stand establishment. A field trial revealed significant heterogeneity among lines in plant height at maturity (Fig. 3A), time of fruit formation and maturation, duration of the crop vegetation cycle and days to full flowering (Fig. 3B, C), number and size of panicles (Fig. 3D-F), grain yield (Fig. 3G), and 1000-seed weight (Fig. 3H). The top-performing Q3 and Q5 (Fig. 3G, H) was early maturing (101-116 days), and Q2 was late maturing (> 136 days) (Fig. 3B). The seed yield ranged from 177.4 g m^{-2} (Q2) to 448.1 g m^{-2} (Q5), with an average of 313 g m^{-2} (Fig. 3G). The significant variability in grain yield was due to the differences in seedling survival and plant growth. As is shown in Fig. 3H, the 1000-seed weight was highest in Q5 (up to 3.15 g) and lowest in Q2 (only 1.28 g) and did not differ significantly between Q5 and Q3.

Fruit and seed morphotype heterogeneity: ImageJ analysis detected distinct differences in seed color (light and brown or dark), shape, and sizes within harvested in KES lines. The percentages of dark (9-17%) and light (83-91%) seeds differed significantly ($p\leq0.05$) within each line (Fig. 4A). The size of light and dark fruits was the same within the lines (Fig. 4B), except for Q3, in which large light fruits exceeded large dark ones in diameter. Small dark brown seeds were predominant in late-maturing plants with low seed productivity. The dark and light seed groups were each further divided into four groups according to their diameter (Fig. 4B, C). Light seeds 2.0-2.5 and 2.5-3.0 mm in diameter were most frequent. Light seeds with a diameter of below 2.0 mm were frequent in the Q2 line (18%), whereas those with a diameter above 3.0 mm were characteristic of Q4 (17%) (Fig. 4B). Similar trends were observed for dark seeds (Fig. 4C). For convenience, instead of four size groups, we decided to use two: small (0.6-2.5 mm) and large (> 2.5 mm). Q4 had the largest seeds, both light and dark ($p\leq0.05$; Fig. 4D).

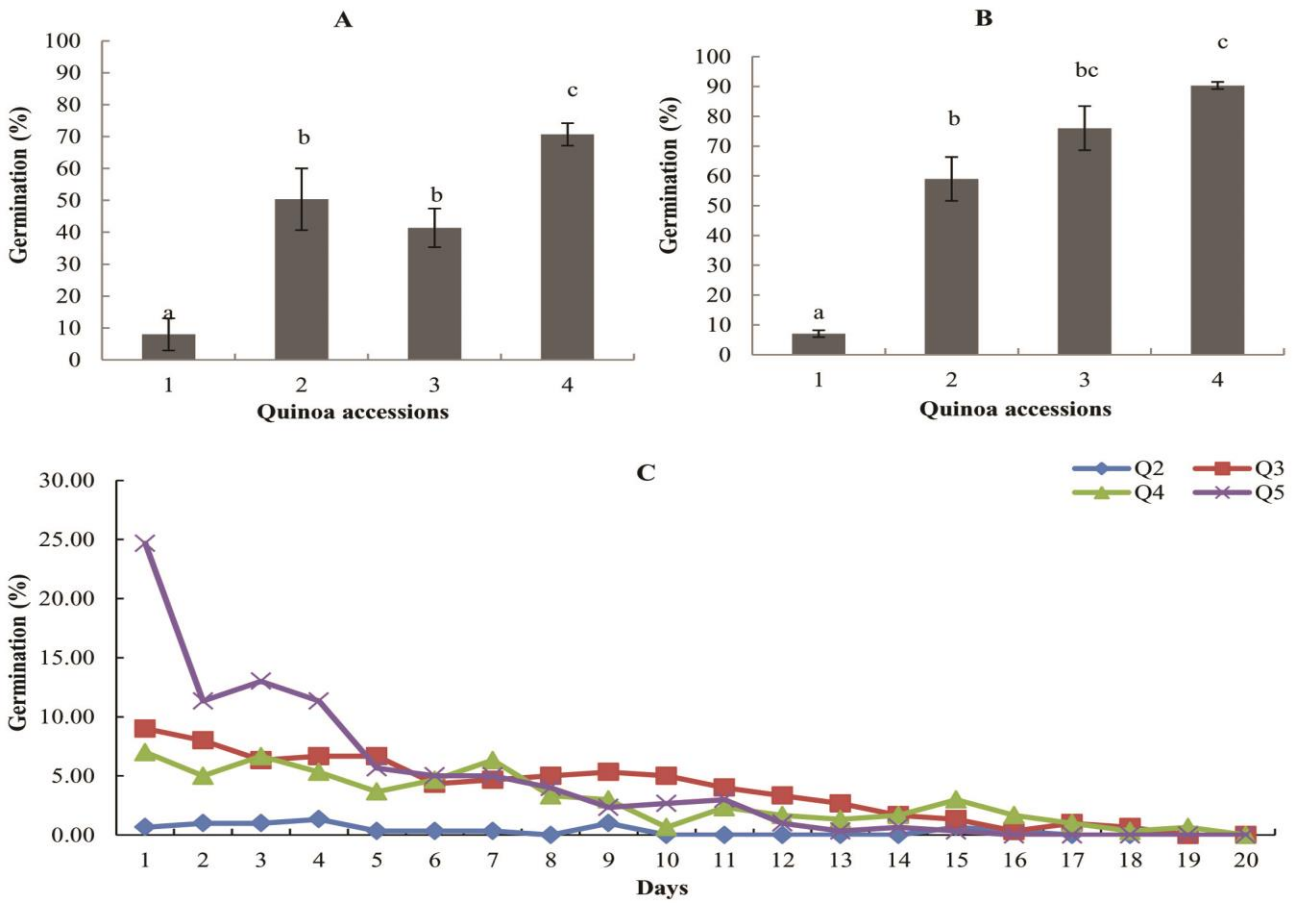

Fig. 2. Energy of light medium-size seed germination of quinoa (*C. quinoa* Willd.) Q2-Q5 lines. (**A**) Seedling emergence in a field trial; (**B**) germination in the laboratory; (**C**) energy of seed germination rate per day in the laboratory. Values are means ± standard errors of three replications. Different letters show significant differences between means at $p \leq 0.05$ (Tukey test).

Q3 and Q5 had intermediate seed diameters among the lines (Fig. 4). Heteromorphic seeds germinated in different ways or in the same way, depending on the line (Fig. 5). The results showed that the germination of the dark seeds was significantly higher than that of the light seeds in Q3. The germination was worse in the dark that in the light seeds in Q4. In Q2 there was no difference in seed germination between light and dark seeds and both were below 10%. In Q5, the germination of dark-large seeds was lower than the light seeds but the germination of the dark-small seeds was higher. Total germination of dark dimorphs (large and small) may be similar within the line as in Q2 and Q3, or different as in Q4 and Q5 (Fig. 5). In Q4 only large dark seeds germinated.

Q5 stood out for its relatively homogeneous large seeds and highest total germination rate of small dark seeds ($p \leq 0.05$). In Q3, dark seeds germinated better than light ones ($p \leq 0.05$) (Fig. 5). Q3 and Q5 had higher germination rates of light and dark seeds than Q2 and Q4 ($p \leq 0.05$) (Fig. 5).

In late-maturing Q2, seeds of different shapes and colors sprouted inside panicles prior to harvest.

Analysis of micromorphology of heteromorphic fruits and seeds by using scanning electron microscopy (SEM): The quinoa lines had dry, upper lysicarpous, single-seeded fruits (Fig. 6A, C-E), sometimes enclosed in a persistent five-lobed perianth (Fig. 6A). Fruits were depressed (dorsoventrally flattened), with nearly circular outlines, an oval lateral appearance, remnants of stylodia at the apex, and a scar at the base (Fig. 6D), sometimes with a short pedicel. Their surface was fine reticulate-foveolate, smooth and radially folded on the lower side, and the margin was slightly festooned. The largest folds were found around the funiculus and seed hilum (Fig. 6D), which were depressed; their position was often marked by a small notch on the periphery of the fruit with a radially oriented shallow groove. The pericarp of *C. quinoa* is strongly adhered to the seed coat. In the Q2-Q5 lines, the pericarp was partly separated in some fruits and a few seeds were free from the pericarp. The surface of the perianth, preserved in some fruits (Fig. 6A, B), was rough, mostly without definite sculpture, but reticulate or papillose-like in some places. Flat compressed structures covered with wax were infrequent (Fig. 6B); they consist of vesicle-shaped hairs, deformed when the fruit was dried.

The two-layered pericarp was filmy, colorless, and dry in mature fruits. On their dorsal side, the exocarp had an alveolar, and in some places papillose, primary sculpture, which was similar in small and large seeds (Fig. 6C a,b, F); on the ventral side, the surface was reticulate (Fig. 6D). The outer walls of the exocarp cells were mostly concave, regardless of the fruit size (Fig. 6C, F). These cells had a thin wall, and a thin mucilage network was clearly detectable inside the cells.

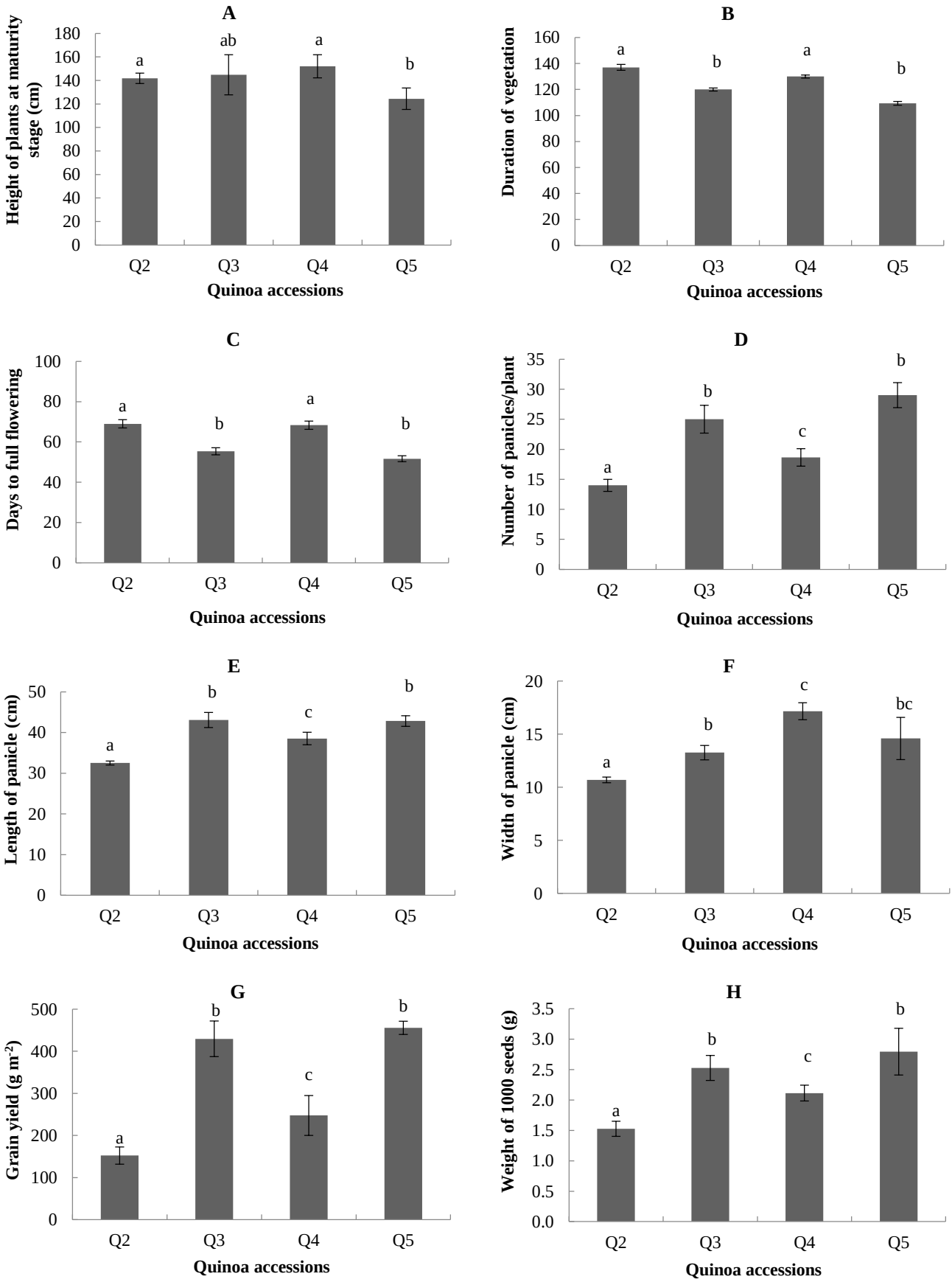

Fig. 3. Agronomic trait variability among Q2-Q5 grown on saline alkaline soils. (A) Plant height at maturity; (B) number of days to maturity; (C) days to full flowering; (D) number of panicles per plant; (E) panicle length; (F) panicle width; (G) grain yield; (H) 1000-seed weight. Values are means ± standard errors of experiments in three replications. Different letters show statistically significant differences between means at $p \leq 0.05$ (Tukey test).

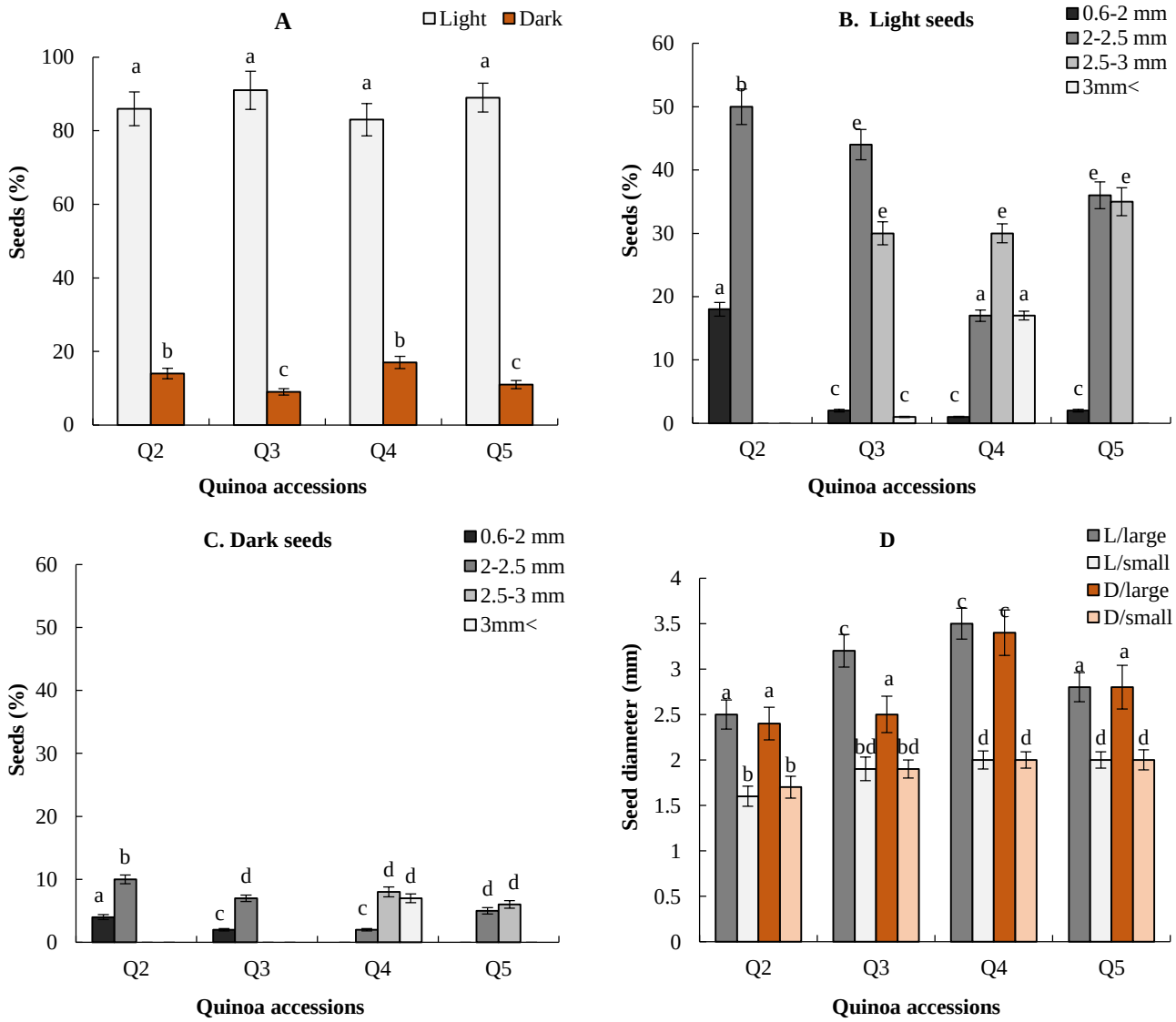

Fig. 4. Phenotypic diversity of seeds in quinoa genotypes. L/large, light large seeds; L/small, light small seeds; D/large, dark large seeds; D/small, dark small seeds. Values in **B** and **D** indicate seed diameter. Values are means ± standard errors of three replications.

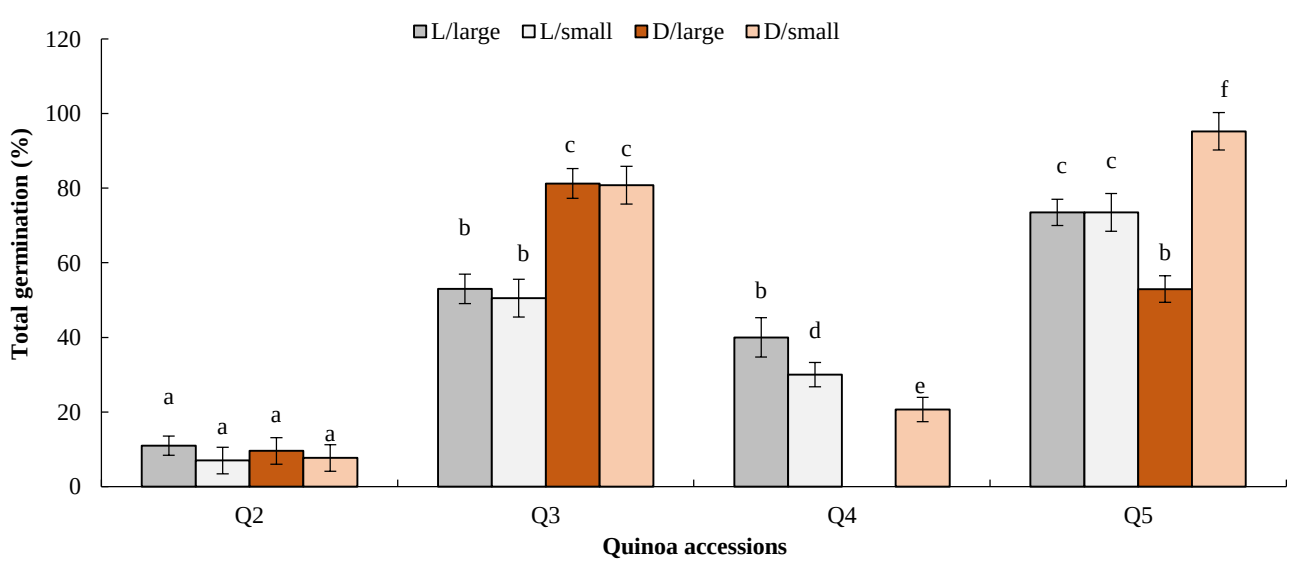

Fig. 5. Germination rates of four seed dimorphs differ in size. L, light; D, dark. Values are means ± standard errors of three replications. Different letters show statistically significant differences between means at $p \leq 0.05$ (Tukey test).

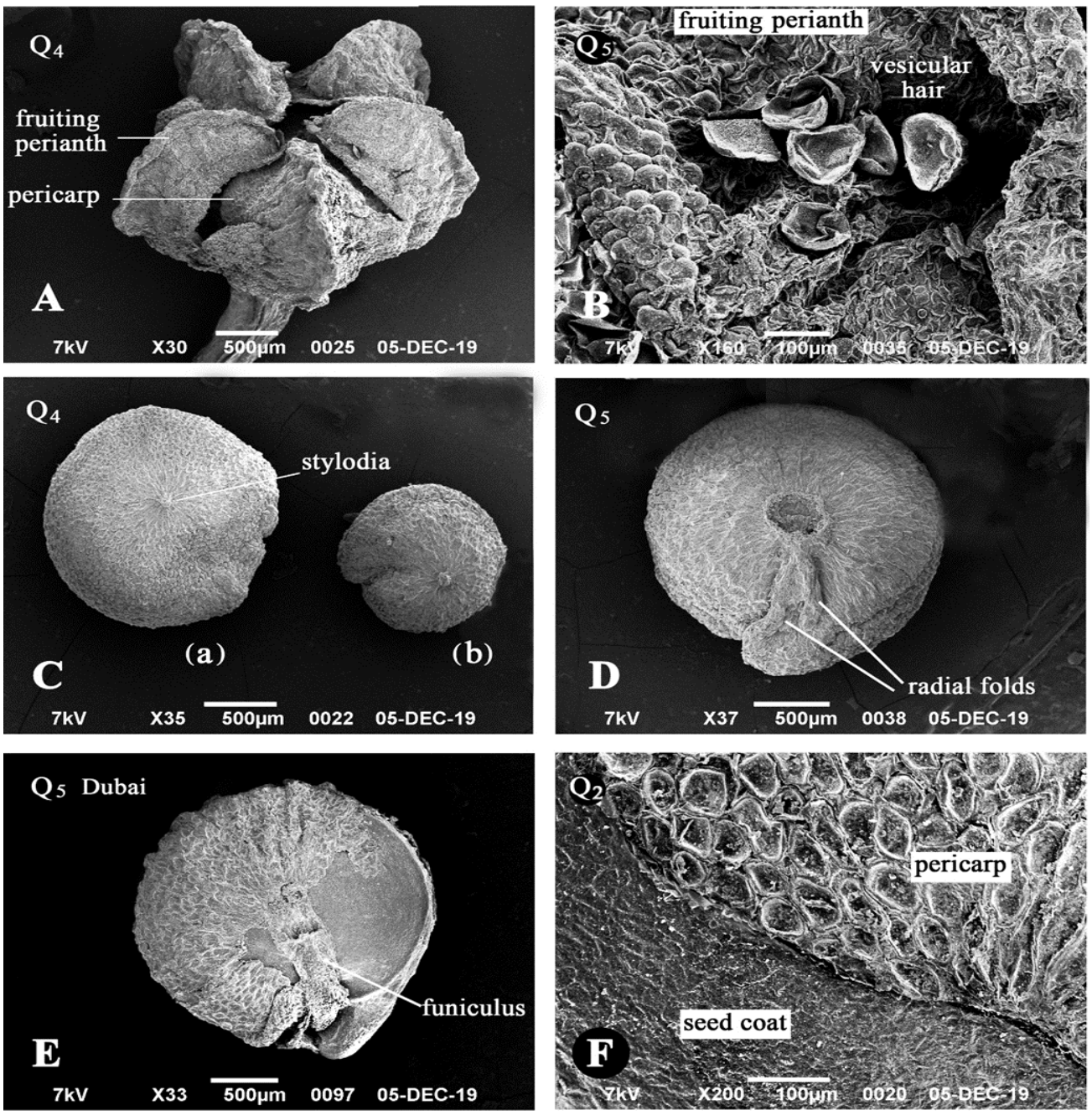

Fig. 6. SEM micrographs of fruits and their surfaces in quinoa accessions. (A) Fruit enclosed in perianth. (B) Fruiting perianth surface with dried vesicular hairs. (C) Large light (a) and small brown (b) fruits from the upper side. (D) Fruit from the bottom side, with radial folds in the funiculus region. (E) Fruit with a partly removed pericarp, from the bottom side. (F) Pericarp and seed coat in surface view. All fruits except C(b) are large. Scale bars: 500 µm in A, C-E; 100 µm in B and F.

Seeds were flattened and located horizontally in the fruit, following its shape, and they occupied the entire fruit cavity. Sometimes they were round-oval or comma-shaped with a projecting radicle. They had a funiculus, which was shaped as a flat strand running between the pericarp and seed coat from the fruit base (Fig. 6D) to the seed hilum located in the seed groove between the ends of the radicle and cotyledons. The protruding radicle of light seeds was white or blackish.

The surface of the seed coat (Figs. 6F, 7) did not differ among quinoa lines and within the same line. The seed coat was flat and rough, often with a faintly discernible or, occasionally, more relieved sculpture (Fig. 7B). The primary exotesta relief was mostly indistinctly shallowly reticulate or irregularly reticulate (Figs. 6F, 7D), with slightly protruding anticlinal and concave outer periclinal cell walls, in places reticulate–areolate with no protruding anticlinal cell walls (Fig. 7E) or smooth-colliculate (Fig. 7F) with depressed anticlinal and smooth or slightly convex outer periclinal cell walls. The secondary sculpture of these cell walls was relieved, smooth-colliculate, with distinct or indistinct tubercles and ribs.

Using SEM, the anatomical structure of seed covers (pericarp, seed coat, inner cuticle) and reserve tissues of the seed (perisperm, endosperm) were described in the example of small dark seed of Q2 line (Fig. 8A-D).

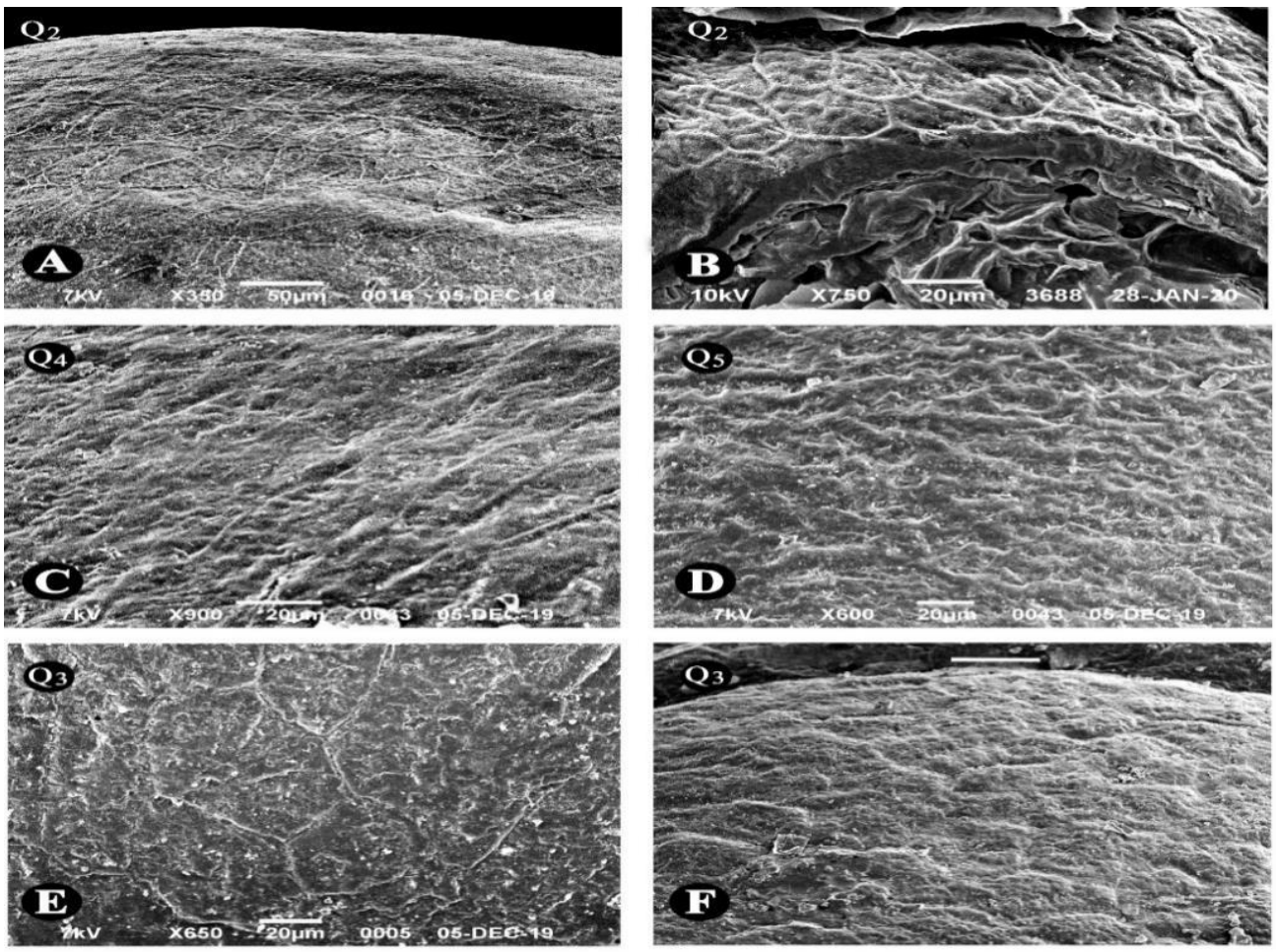

Fig. 7. SEM micrographs of the seed coat surface in quinoa accessions: small seeds (A-D) and large seeds (E, F). Scale bars: 50 µm in A and F, 20 µm in B-E.

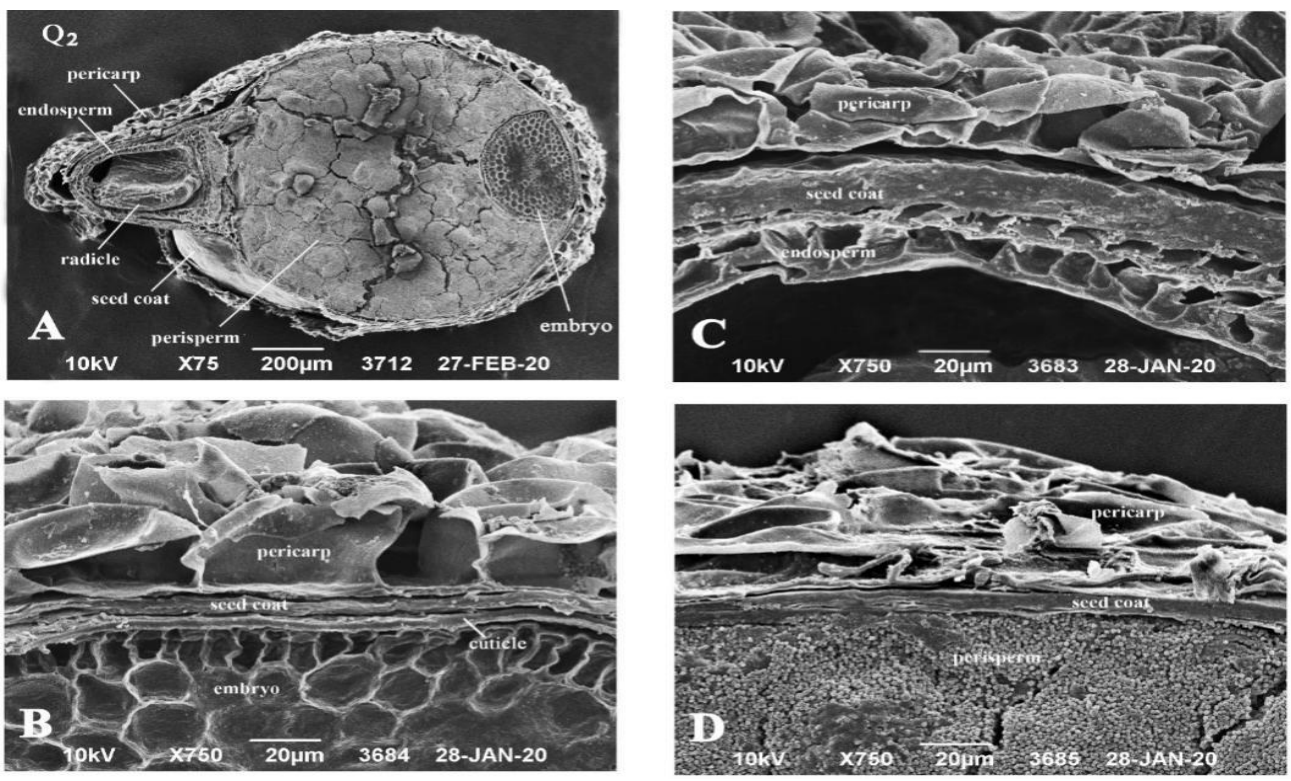

Fig. 8. SEM micrographs of longitudinal median section of Q2 small dark seed. (A) section of a whole seed. (B) section portions in hypocotyl region. (C) in radicle region. (D) in side region. Scale bars: 200 µm in A, 20 µm in B-D.

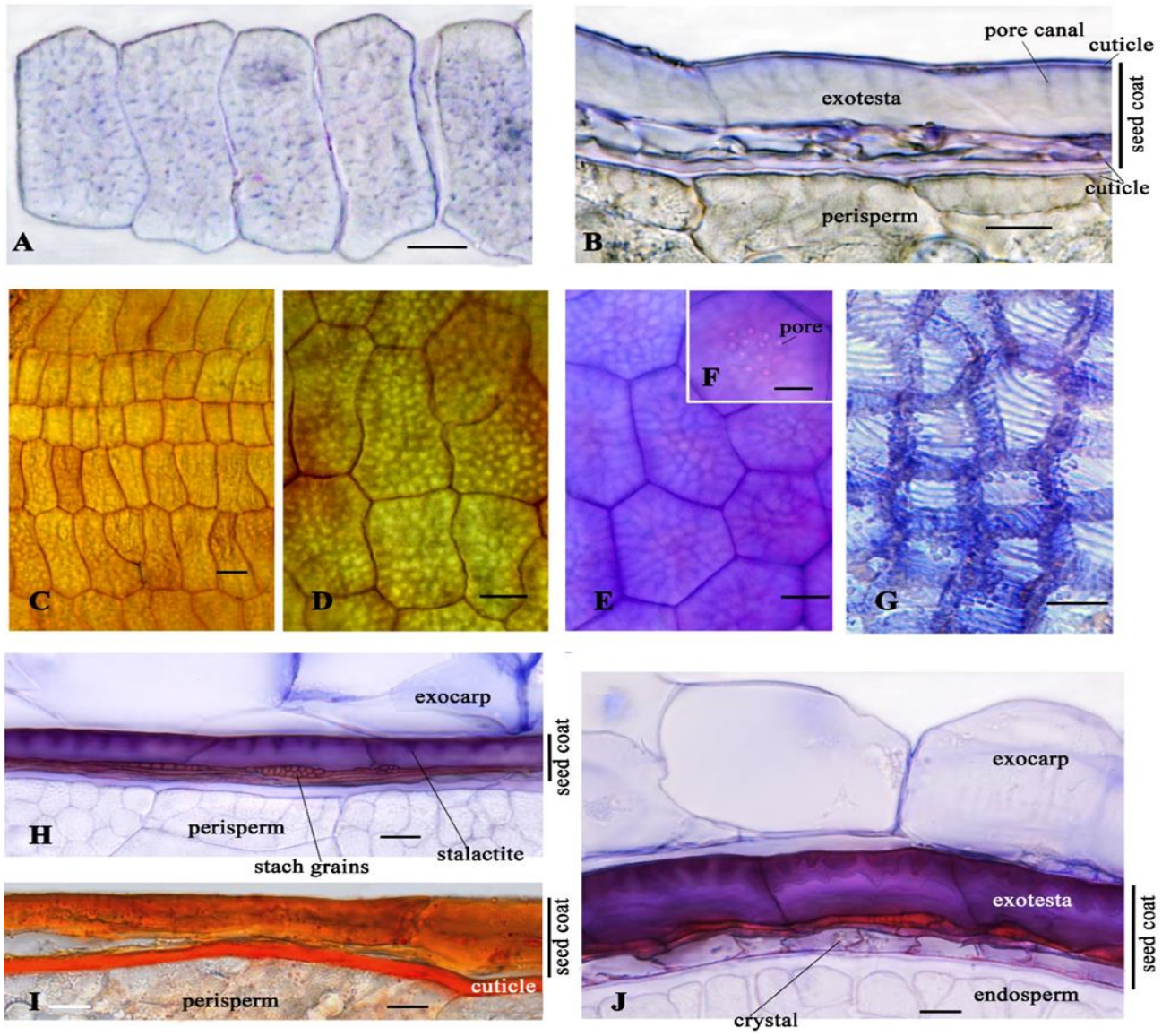

Fig. 9. Seed coat structure of heteromorphic seeds in the Q2 line (light microscopy). (A, B) Large light seeds. (C-J) Small dark seeds. (A) Exotesta cells in surface view (SV). (B) Longitudinal section (LS), lateral side of the seed. (C) SW, exotesta cells on the dorsum of the seed. (D, E) SW, exotesta cells on the lateral side of the seed. (F) SW, magnified portion of E. (G) SW, endotegmen. (H, I) LS, lateral side of the seed. (J) LS, radicle region. Staining: A, B, E-H, J, Gentian violet; C, D, none; I, Sudan IV. Scale bars: 20 μm in A, C-E; 10 μm in B, F-J.

Study of anatomical variation of seed envelopes in Quinoa using light and transmission electrone microscopes: The light microscopy of quinoa pericarp and seed coat (Fig. 9A-J) revealed that exocarp cells had a thin wall, and a thin network of mucilage substances inside the cells (Fig. 9J). The exocarp outer periclinal walls, being mainly concave in dry intact fruit, became convex when wet (Fig. 9J), as the cells quickly accumulated water, apparently owing to the presence of mucilage in the cavity. The exocarp cells clearly contained mucilage.

The seed coat was leathery, glossy, and white or yellowish to light brown in light seeds (Fig. 9A, B) and brown in dark seeds (Fig. 9C, D), exotesta-endotegmic, formed mostly by the strongly thickened outer periclinal cell walls of the exotesta (Fig. 9B-D, H-J).

In light large seeds the exotesta cell length was 90-115 μm. The cells' uncolored translucent outer periclinal wall (Fig. 9A, B) was up to 18 μm thick and pierced with vertical and oblique narrow cavities (probably pore canals). It did not stain with phloroglucinol (data not shown) or gentian violet (except for very slight staining of a narrow canals area), indicating an almost complete absence of lignin. In dark small seeds (Fig. 9C-I) the exotesta cell length was 45-95 μm, the outer periclinal cell wall was thinner (up to 12 μm) and pierced with vertical narrow cavities; pronounced short, dark, narrow-triangular bands, usually named "stalactites", were visible in the sections (Fig. 9H, J). Tannins that impregnated the cell walls of the exotesta and were contained in cell cavities gave the dark brown coloration to the seed coat (Fig. 9C, D). In surface view, the tannin-stained parts of the outer wall formed a reticulate pattern (Fig. 9C-E), with pores in the center of areoles (Fig. 9F). The exotesta cell walls of dark seeds were positive for lignin when stained with gentian violet (Fig. 9E, F, J) or with phloroglucinol and sulfuric acid (data not shown). In the

region of the protruding black root of the embryo, which was present in dark and some light seeds, the exotesta cell walls also contained lignin, and stalactites in the outer periclinal walls were more pronounced than in other seed areas (Fig. 9J).

The endotegmen in mature seeds was strongly compressed and poorly distinguishable in sections, but it was clearly visible in surface view (Fig. 9G). In seeds of both types, tracheid-like cells of this layer were small and polygonal, with scalariform thickenings of the thin inner periclinal and anticlinal cell walls.

Exotesta cells were polygonal, often hexagonal, and smaller in small seeds, and they were covered with a thin cuticle. On the sides of the seed the cells were broad and polygonal or irregularly polygonal with curved anticlinal walls, without definite orientation (Fig. 9D, E). On the dorsum of the seed and adjacent parts, the cells were almost rectangular, longitudinally oriented (Fig. 9C) and arranged in at least six rows. The two central rows were small, and the adjacent rows were more elongated. Slit-like cavities of the exotesta cells contained plastids with starch grains (Fig. 9H) and sometimes single crystals and druses (Fig. 9J). The volume of cell content determines the variation in the seed coat thickness. There was a thick (2.5-4.5 μm) cuticle between the endotegmen and perisperm (Fig. 9I); the cuticle was thinner in light seeds than in dark seeds.

Transmission electron microscopy (TEM) studies showed that this cuticle is permeated with many small veins, probably fragments of the dendrite network characteristic of the reticular type of perisperm and endosperm cuticles. The study of the cuticle in those portions of the seed sections, where both perisperm and endosperm were present, showed that it is of nucellar origin. The starch-filled cells of the nucellus (perisperm) are very thin-walled. The outer cells of the endosperm are covered with a thin cuticle.

Table 1. Variation in thickness of the seed envelope of light seeds in quinoa lines.

Quinoa Line	Large seeds		Small seeds	
	Thickness (μm)	Mean value	Thickness	Mean value
Q_2	11-29	20.1±0.25	9-23	14.8±0.30
Q_3	13-29	19.5±0.19	9-23	14.5±0.19
Q_4	11-26	19.3±0.22	9-29	16.9±0.20
Q_5	14-29	19.3±0.09	11-23	16.4±0.20

The differences in seed size were accompanied by differences in the thickness of protective seed envelope (SE = seed coat + underlying cuticle). The SE in all lines was always thicker in large light seeds than in small light seeds (Table 1). There was weak detectable difference in the SE thickness of the light large seeds among the lines: they refer to the min. and max. values that are not reflected in its average values of 19.3-20.1 μm. Small seeds of different lines, in addition to differences in the max. and min. SE thickness, differed (more significantly than large seeds) in their average value: 14.5-16.9 μm. It is higher for Q4 and Q5 than for Q2 and Q3. Q4 had the smallest difference in SE thickness between light large and small seeds, and Q2 the largest one.

As is shown in Table 1 the largest average (20.1±0.25) seed envelope thickness was characteristic for Q2, which had smaller light fruits and seeds with lowest percentage and energy of germination compared to other lines.

Discussion

The pseudocereal halophyte *C. quinoa* has attracted interest worldwide owing to its tolerance to harsh environments and the valuable nutritional properties of its seeds (Nowak *et al*., 2016; Nanduri *et al*., 2019). Native to the Andes mountains, it has been cultivated from Colombia to Argentina and Chile for over 7,000 years. Quinoa has been recently introduced into the agricultural practices of the Aralo-Caspian drylands, where soil salinization is a serious threat (Yamanaka & Toderich, 2020; Mamedov *et al*., 2020). Quinoa genotypes can produce high-quality seeds under a wide range of climates and salinity stresses in which conventional crops cannot grow (Choukr-Allah *et al*., 2016; Jacobsen, 2017; Hinojosa *et al*., 2018; Khaitov *et al*., 2020, Ilham Abidi *at al*., 2022).

The quinoa fruits investigated in this study were classified as upper lysicarpous nuts (Bobrov *et al*., 2009) or nut-like fruits (Butnik, 1991). The quinoa lines produced heteromorphic fruits and seeds, which have different germination behavior under stress environments. Heteromorphism of fruits and seeds in size and color is noted for all lines grown in KES, which distinguishes them from those harvested in Dubai, in which both large and small seeds were equally light, but the seed coat is often had brown spots. The appearance of heteromorphism in harvested in KES *C. quinoa* fruits and seeds is apparently adaptive in nature and is a response to stressful growing conditions (high salinity and drought), which are more severe than in Dubai. Fruits varied within the line and between lines in size and in color, seeds varied in shape, size, color, thickness and chemical composition of the seed coat. Our findings are consistent with previous *Chenopodium* species reports, which also described morphologically distinct diaspores (Sukhorukov, 2014; Varriano-Marston and De Francisco, 1984). Many of the annual species of the Chenopods (*Chenopodium, Salsola, Salicornia* representatives) belongs to halophytes (salt-loving plants), for which the development of heteromorphic fruits and seeds is common (Toderich *et al*., 2009, Sukhorukov, 2014; Butnik *et al*., 2016, Maliro & Njala, 2019, Shuyskaya *et al*., 2021). This adaptive feature is accompanied by the appearance of a number of dissemination modes and diversities in seed germination characteristics (Kadereit *et al*., 2007).

SEM microphotographs of quinoa lines confirmed the conclusion (Devi & Chrungoo, 2015) that a smooth surface and weakly expressed sculpture of the seed coat are typical of quinoa seeds. The recorded diversity of sculpture from indistinct shallow-reticulate to smooth-colliculate might be caused by variation in the shape of the outer periclinal walls of exotesta cells from slightly convex to slightly concave within the seed. The

flattened-colliculate sculpture with submerged anticlinal and flat or slightly convex outer periclinal cell walls of the exotesta cells likely corresponds to the smooth-tuberculate sculptural type in quinoa (Karcz *et al*., 2005). Our observations also agreed with Karcz *et al*., (2005) description of the tuberculate secondary sculpture of the seed coat surface and the alveolate surface of the quinoa fruit.

We confirmed the data for the *Chenopodium* genus (Kowal, 1953; Prego *et al*., 1998; Sukhorukov & Zhang, 2013) that the seed coat consists of a sclerified exotesta and remnants (cell walls) of two obliterated inner layers of the integument. According to Abdelbar (2018), the two layers of the seed coat may maintain the moisture level required for the embryo and perisperm and make the seed hard. We assume that three structural elements make up the multi-level water-bearing and water-retaining system in the quinoa fruit. They are: (i) the exocarp with its thin-walled cells, within which a thin network of mucilage is noted, probably deposited in the vacuole; these cells swell very quickly when soaked, gaining water; (ii) the exotesta cells, the walls of which are pierced with pores; through these pores water may also enter the seed; and (iii) tracheid-like endothegmen cells, associated with the conductive bundle of the seed, ending in the chalaza (point where the ovule is attached to the fruit). These water-conducting cells enable moisture to spread throughout the seed. Tracheid-like cells have been observed in the pericarp and seed coat of various groups of flowering plants (Werker, 1997; Kravtsova, 2006). These cells are thought to function in water penetration into the seed, transport, and accumulation in the seed coat. The structures listed above ensure rapid water penetration into the quinoa seed, stimulating speedy germination.

Many seed storage substances, such as tannins and trypsin inhibitors, are found in the seed coat of quinoa and may have detrimental effects on seed germination and food quality (Prego *et al*., 1998; El Hazzam *et al*., 2020). Quinoa grain quality is decreased by anti-nutritional saponins, a terpenoid class of secondary metabolites that are deposited in the seed coat and compromise the successful commercialization of quinoa grains at global market (El Hazzam *et al*., 2020, Mhada *et al.*, 2020). Tannins, and lignin detected by us in quinoa lines, are thought to increase seed coat hardness (Boesewinkel & Bouman, 1984), consistent with seed dormancy and seed germination traits (Butnik *et al*., 2016)

Heteromorphy allows diverse seeds of the same species or even the same cultivar to germinate at different times and under different temperature and salt regimes (Butnik *et al*., 2016,); this is particularly important for seedlings emergence and plant survival under extreme conditions (Gutterman, 1994). The appearance of dark seeds in quinoa is likely an ancient trait, as there is evidence that originally the seeds of *C. quinoa* were dark and light ones appeared as a result of acclimatization and advanced breeding under new environmental conditions (Wungrampha *et al*., 2020). *C. album* develops more light seeds under severe soil salinity; they are more resistant to salinity and germinate rapidly without dormancy under a wide range of conditions (Yao *et al*., 2010, Tanveer & Shah, 2017). In quinoa cultivars such as Chadmo and Titicaca, delay in germination is stronger in seeds with a darker coat (Ceccato *et al*., 2015).

In halophytes, production of dormant seeds is a mechanism for survival under adverse environments: small dormant seeds remain in the soil until salt concentration permits seed germination (Wang *et al*., 2012a, b). In *Suaeda salsa*, brown seeds have higher germination rates and absorb water better than black seeds (Li *et al*., 2015). We showed that, as the growing season progresses, the germination rate of all types of seeds decreases (Fig. 10A). At the same time, the similarity of light seeds, both large and small, decreased almost equally, and among dark seeds there was a scatter in the rate of germination of large and small seeds during the growing seasons. There was also a positive relationship between seed germination and grain yield (Fig. 10B).

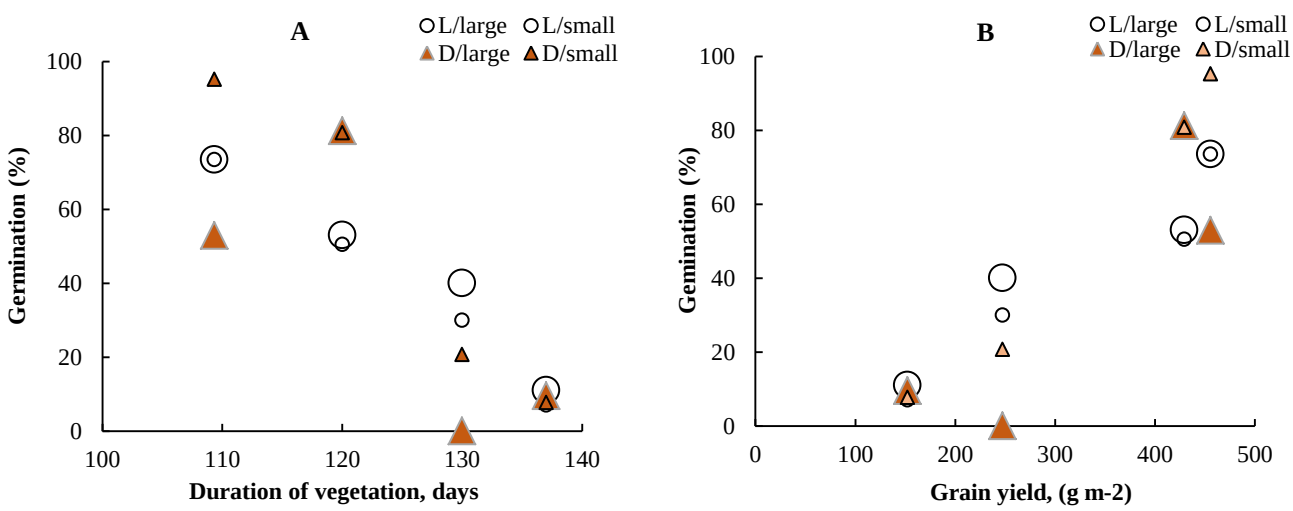

Fig. 10. Germination of different seed dimorphs in quinoa genotypes in relation to (A) duration of vegetation and (B) grain yield. L/large, light large seeds; L/small, light small seeds; D/large, dark large seeds; D/small, dark small seeds.

Unlike other species, such as *C. album*, no positive relationship was found between seed color and total germination or duration of dormancy period. Germination rates of dark seeds may be better than those of light ones (Q3), or worse (Q4), or the same or about the same (Q2, Q5). However, it is hard to explain the highest germination percentage of small dark seeds in Q5. Total germination rates of dark dimorphs (large and small) may be similar within a line (Q2 and Q3), or differ (Q4 and Q5). The size of light and dark fruits was the same within the lines, except for Q3, in which large light fruits exceeded large dark ones in diameter. The difference in the thickness of the protective seed envelope (seed coat + inner cuticle) in light large and small seeds within all lines had almost no effect on their germination, except in Q4, where large light seeds germinated a little better than small ones. In less salt-resistant late-maturing Q2 and Q4 lines we observed an enhancement of seed heterogeneity: in both color and size, with an increase in the average seed size (Q4), or only in color, with a decrease in the average seed size (Q2). Seed heteromorphism in these two lines was accompanied by low levels of seed productivity and delay or unhomogenous seed germination in the field. It may be assumed that Q2 and Q4 display different "expectant" survival strategies under new cultivation conditions. The studied lines differed slightly in the thickness of the protective seed envelope (seed coat + inner cuticle) of light seeds. The largest average thickness was characteristic of Q2, which had smaller light fruits and seeds and the lowest percentage and energy of germination of all the lines.

We found that one of the features distinguishing dark brown quinoa seeds from the predominant light seeds was the presence of short, poorly developed stalactites. Stalactites have not previously been noted in quinoa. The appearance and structure of these inclusions in the exotesta cell wall during plant ontogeny have been observed in some representatives of Chenopodiaceae, Amaranthaceae, and taxonomically related Nyctaginaceae (Kowal, 1954; Butnik, 1991; Sukhorukov *et al*., 2015; Veselova *et al*., 2016). *Amaranthus* stalactites are solid masses of tannins and phytomelanins; they fill vertical cavities in the cell wall, produced from its lysis (Dzhalilova *et al*., 2015). In contrast to pore canals, which always end with a pore-closing film (i.e., the primary cell wall) (Esau, 1969), stalactite cavities at all stages of formation are isolated from the primary cell wall and plasma lemma, which exhibits morphogenetic activity. The fine structure of the cell wall with stalactites and the mechanism of stalactite formation have not yet been studied in detail. This is especially interesting in quinoa, as the appearance of dark seeds is likely an ancient trait (Heiser & Nelson, 1974; Sukhorukov & Zhang, 2013).

The Quinoa seeds investigated during this study have revealed that differences in seed size were accompanied by differences in the thickness of protective seed envelope (SE = seed coat + underlying cuticle). The SE in all lines was always thicker in large light seeds than in small light seeds. According to Sukhorukov (2014), the structure of cuticle in the fruit and seeds of Chenopodiaceae is always well defined and thick. However, Varriano-Marston and DeFrancisco (1984) studied the quinoa seed coat with TEM and refer to this layer as cutin-like; Van Raamsdonk *et al*., (2010) believe that it is a remnant of the endosperm. On the basis of TEM data, Yakovleva (2002) concluded that the inner cuticle in quinoa seeds is of the reticular type and is permeated by thin electron-dense veins. We concluded that the thick inner cuticle in quinoa seeds belongs to the nucellus. However, we do not know if seed cuticle has an influence on seed germination or other seed physiological processes. Our finding is consistent with the report by E. Imbert (2002), who suggested that structural differences between seed types in some species of Asteraceae is related with differences in pericarp and embryo organ structural differentiation, which in return determines the seed germination rate. Seed coat thickness may control germination by preventing radicle protrusion and regulating water uptake. Differences in pericarp structure as it was described by E. Imbert (2002) are responsible for divergent germination in heterocarpic species of Asteraceae (e.g., *Anthemis chrysantha*, *Heterotheca subaxillaris*, and *Hemizonia increscens*).

The pigmentation and structural integrity of the seed coat are important for its permeability (Debeaujon *et al*., 2000). Unlike *C. album*, quinoa has no positive relationship between seed color and germination rate, under labotratory or field conditions. There is no strict accordance between the thickness of the protective seed envelope in light large and small seeds and their germination rate. The potentially useful lines Q3 and Q5 had lower percentages of dark seeds than Q2 and Q4, but their germination rates were the same or even better. The best line Q5 had relatively homogeneous large seeds and the highest germination percentage of small dark seeds. The Q2 and Q4 lines produced fewer seeds with poor germination, and had higher heterogeneity in color and size, with an increase in the average seed size (Q4), or in color only, with a decrease in the average seed size (Q2).

The importance of seed heterogeneity studies has been highlighted by various authors, since characteristics such as weight and size allow the differentiation of seeds for more homogeneous grain yield, enabling greater uniformity and the improvement of seed emergence and vigor in the face of salinity and heat stresses (Hirich & Choukr-Allah, 2020, Ilham Abidi *et al*., 2022). Differences in seed size in the Q2-Q5 lines may be a consequence of maturation since quinoa seeds are at different maturation stages in the same plant and even within the same panicle. Our findings confirm the findings of Rodrigues *et al*., (2020), who showed the importance of seed morphology diversity in the seed dispersion and seedling establishment of quinoa introduced less than 30 years ago in Brazil. In *C. album*, *C. berlandieri*, and *C. bonus-henricus*, the seed coat of dark seeds is associated with strong dormancy (Dorne, 1981; Halwas, 2017). The average seed coat thickness of small dark seeds is only slightly greater in Q5 than in Q2-Q4. A peculiarity of late-maturing Q2 and Q4 that distinguished them from other studied quinoa genotypes was mass seed sprouting inside the panicles before harvest.

Salinity stress may affect seed production and the formation of different seed heteromorphs with different rates of germination and seedling survival (Ashraf *et al*., 2009; Chedlly *et al*., 2008; Hasanuzzaman *et al*., 2019; Ozturk *et al*., 2006, 2018, 2019, 2023). This has been particularly reported in various species of Amaranthaceae (Wang *et al*., 2015, 2018; Cai & Gao, 2020; Khaitov *et al*., 2020). *Atriplex centralasiatica* seedlings from yellow seeds grow better than seedlings from brown seeds under salinity stress (Xu *et al*., 2011). Excess soil salinity, as we have already described (Toderich *et al*., 2020), induces interline differences in quinoa growth, fruit chemical composition, and seed nutritive properties. The seed coat is usually thinner in non-tolerant seeds than in salt sensitive seeds (Serrato-Valenti *et al*., 1990; Liu *et al*., 2018); its thickness also depends on the accumulation of phenolics and other chemical substances in the palisade cells. However, it is still unknown how these substances interact during seed germination; therefore, physiological, and molecular data on seed coat development in heteromorphic seeds need to be explored in the future investigations. Variation of seed heteromorphism in quinoa and the effect of changing or new environments on grain yield in plants with dimorphic seeds need further investigation.

Conclusions

Our results show that the presence of dark seeds in *C. quinoa* lines grown at the Kur-Araz lowland of Azerbaijan (KES) distinguishes them from the original source seeds from Dubai, in which all seeds are equally light, but the seed coat often has brown spots. Light seeds were predominant (83-91%) in *C. quinoa* lines from KES.We are positing that this peculiarity of *C. quinoa* seeds grown at KES might be a response to the harsh cultivation environment (e.g. strong salinity and drought). We are assuming that heteromorphism of *C. quinoa* seeds collected at KES might be a response to the changing cultivation environments (strong salinity and drought). The dark color of the seeds is due to the tannins containing in the exotesta walls and cell lumen. Histochemical analysis indicated also the presence of lignin in the exotesta of dark seeds. The presence of tannin-containing seed coat spots in the radicle area only in some of the light seeds further increases seed diversity.

Some new information on the quinoa seeds structure obtained: the pattern of the seed coat micromorphology was similar in the heteromorphic seeds, with weak surface sculpture; the thick cuticle underlying the seed coat is most likely of nucellar origin, it is characterized by reticulate ultrastructural type; stalactites that have not previously been noted in quinoa seeds, were discovered in the exotesta of dark seeds.

Differences in seed size, color, seed coat anatomical structure, histochemistry, metabolite amount, as well as in total germination percentage between different seed fractions indicate multiple strategies of dispersal and germination in quinoa lines to ensure survival under stressful conditions of KES. Based on variation in the ontogeny and structure of the seed coat and seed reserve substances among seed morphotypes in quinoa lines, we conclude that the large light seeds produce potentially more competitive seedlings than do dark small seeds. Synchronized seedling emergence and stand establishment, coupled with the exceptionally high grain yields of the Q3 and Q5 lines, demonstrated their good adaptation to new cultivation environments. The Caspian arid region could be considered as a potential area for cultivation of high-performing quinoa genotypes, the seed functional features of which were not affected by abiotic stress.

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