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Drought-Induced genomic and epigenetic variations in Quinoa genotypes revealed by iPBS and CRED-iPBS marker systems

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Drought stress significantly impacts crop productivity, yet its influence on genomic and epigenetic variation in quinoa remains poorly understood. This study aimed to assess DNA damage and cytosine methylation alterations in six quinoa genotypes (Titicaca, Rainbow, Moqu Arrochilla, Cherry Vanilla, China, and White) exposed to five irrigation levels (5%, 10%, 25%, 50%, and 100% field capacity). Genomic changes were evaluated using inter-primer binding site (iPBS) markers, while DNA methylation was analyzed via CRED-iPBS. Results revealed genotype-specific polymorphism and genomic template stability (GTS) responses to irrigation stress. Moqu Arrochilla showed the highest GTS (84.6%) under 5% field capacity, while White exhibited the lowest (35.0%) at 50% field capacity. CRED-iPBS analysis indicated both hyper- and hypomethylation events depending on stress intensity, with China and Moqu Arrochilla genotypes displaying the highest polymorphism rates for MspI (42.9%) and HpaII (39.0%), respectively. These findings highlight the drought-induced genomic and epigenetic variability in quinoa, emphasizing the utility of iPBS and CRED-iPBS techniques for screening stress-responsive genotypes. This work contributes to the understanding of stress adaptation and may inform breeding programs targeting drought tolerance.

Agriculture is not only a primary source of food and employment but also a vital component of national economies, making its sustainability a global priority[1]. However, the productivity and quality of cultivated crops are heavily influenced by local meteorological conditions, with water availability being one of the most limiting factors[2]. The ongoing challenges posed by global climate change complicate the ability of plant breeders to anticipate and address the obstacles to future food security[3,4]. Among these challenges, drought is considered one of the most critical abiotic stressors, as it substantially reduces crop yields. In plants, drought disrupts key physiological processes, including respiration, photosynthesis, and stomatal function, thereby impairing overall growth and metabolic activity.

In response to drought stress, plants initiate a range of adaptive mechanisms, including morphological and structural changes, the activation of drought-responsive genes, hormone biosynthesis, and the accumulation of osmoprotectants[5]. Among crops recognized for their resilience to such stresses, quinoa (Chenopodium quinoa Willd.) has garnered increasing attention due to its exceptional nutritional profile and remarkable adaptability to adverse environmental conditions[6]. As a gluten-free pseudo-cereal, quinoa is notably rich in high-quality protein, unsaturated fatty acids, fiber, vitamins, magnesium, and iron, rendering it a valuable crop for promoting food and nutritional security in the 21st century[7,8]. Its seeds are widely consumed for their high crude protein content and dietary benefits. In addition to its nutritional value, quinoa exhibits notable tolerance to multiple abiotic stressors, including drought, salinity, heat, and cold[9]. It has demonstrated the ability to germinate, grow, and reproduce even in hyper-arid environments such as those of Chile, northwest Argentina, and the Altiplano region of Bolivia and Peru regions where water scarcity significantly limits agricultural productivity[10]. Despite this recognized resilience, the molecular basis of quinoa's drought tolerance, particularly in terms of epigenetic

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and genome-wide responses, remains insufficiently understood, underscoring the importance of further research in this area.

Quinoa responds to limited water availability through a range of stress avoidance mechanisms that regulate water loss and uptake[11]. These include both morphological and physiological adaptations that enable the plant to maintain growth under drought stress. Notably, under dry conditions, quinoa modifies its root and leaf development, often exhibiting minimal ontogenic variation[12]. In arid or rainfed environments where other crops struggle due to shallow or ineffective root systems, quinoa's ability to restrict leaf expansion contributes to improved water-use efficiency and enhanced drought tolerance. This adaptive response minimizes transpirational water loss and helps sustain plant survival during periods of water scarcity. Under water-deficit stress, quinoa increases solute accumulation, thereby lowering cellular water potential and stimulating root growth[13]. Concurrently, stomatal closure and reduced shoot elongation limit evaporation and further conserve water[14]. Thus, modulation of shoot development, which directly affects the transpiration surface area, constitutes a key component of the drought response strategy in quinoa and other resilient crop species.

Numerous studies have demonstrated that quinoa has evolved adaptive mechanisms to alleviate the effects of drought stress, including high water-use efficiency and an elevated shoot-to-root ratio[15]. Although the association between drought stress and DNA methylation has received less attention than other abiotic stress responses[16], accumulating evidence suggests that water deficit can lead to notable epigenetic changes. For instance, drought stress has been shown to upregulate DNA methyltransferase genes in wheat[17], and to induce genome-wide hypermethylation in upland cotton, which returned to baseline levels upon rehydration[18]. Such stress-induced genomic and epigenetic alterations have been analyzed using a variety of molecular marker systems, including random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), coupled restriction enzyme digestion-random amplification (CRED-RA), and methylation-sensitive amplified polymorphism (MSAP)[19].

Among these, the inter-primer binding site (iPBS) technique is a PCR-based DNA fingerprinting method that does not require prior sequence knowledge. It utilizes primer-binding sites within the long terminal repeat (LTR) regions of retrotransposons and has been successfully applied to assess genome variability and stability in species such as guava, barley, wheat, pear, maize, and apricot[20]. The CRED approach, when combined with iPBS markers (CRED-iPBS), offers a robust method for detecting changes in cytosine methylation under environmental stress. This system uses methylation-sensitive restriction enzymes, particularly HpaII and MspI, to reveal differential methylation patterns at CCGG sequences[21]. These two isoschizomers differ in their sensitivity to cytosine methylation: HpaII cleaves DNA only when the external cytosine is hemi-methylated, while MspI is blocked by both hemi- and fully methylated external cytosines[22,23]. This differential cleavage pattern allows for detailed profiling of drought-induced methylation changes, especially in stress-adaptive crops like quinoa. The modulation of DNA methylation in response to adverse conditions, such as drought, low temperature, and high salinity, is a well-recognized phenomenon in plants [34]. However, there is a scarcity of research aimed at elucidating the impact of restricted water treatment on DNA methylation. The DNA methylation patterns of quinoa plants exhibit significant variations under limited water conditions, including methylation, hypermethylation, and demethylation.

Although quinoa has been widely recognized for its resilience to abiotic stresses such as salinity and drought, the molecular mechanisms underlying this tolerance remain poorly understood. In particular, limited information is available regarding the role of genomic stability and epigenetic modifications, such as cytosine methylation, in response to prolonged drought conditions. Therefore, this study aims to investigate the genomic and epigenetic responses of six quinoa genotypes under varying irrigation levels using inter-primer binding site (iPBS) and coupled restriction enzyme digestion-iPBS (CRED-iPBS) markers. This approach enables the detection of both genomic rearrangements and DNA methylation alterations, offering insight into the molecular basis of drought stress adaptation in quinoa.

Results

Physiological responses A prior study by Akçay and Tan[24] demonstrated that the physiological responses of quinoa to water deficit vary significantly depending on both irrigation level and genotype. Their findings indicated a gradual decline in key growth parameters such as shoot and root lengths, dry biomass accumulation, and drought tolerance indices with decreasing irrigation. Interestingly, an increased root-to-shoot ratio was observed at 50% field capacity, suggesting a compensatory mechanism that enhances water uptake under moderate drought stress. Among the genotypes assessed, Titicaca, Sandoval Mix, Moqu Arrochilla, and Mint Vanilla exhibited notable drought tolerance based on physiological indicators. These results underscore the importance of evaluating morphological traits particularly root and shoot development as reliable indicators of drought-induced stress. Such parameters are not only essential for identifying tolerant genotypes but also for establishing meaningful correlations between physiological responses and subsequent changes in genomic DNA polymorphism and cytosine methylation patterns, particularly under varying irrigation conditions (Table 1).

iPBS analysis The iPBS analysis was performed to assess the genomic polymorphism induced by drought stress across six quinoa genotypes subjected to five different irrigation levels. Notable differences in banding patterns were observed between control (100% field capacity) and stressed plants, including the disappearance of control-specific bands and the appearance of novel bands. Each of the 10 iPBS primers used produced varying numbers of bands depending on genotype: in the control group, Moqu Arrochilla yielded 26 bands, White 40, Cherry Vanilla and Rainbow 50, Titicaca 54, and China 69. Individual primers generated 1–5 bands in Moqu Arrochilla, 2–7 in White, 2–10 in Cherry Vanilla, 1–8 in Rainbow, 1–9 in Titicaca, and 3–12 in China. Under drought conditions, new bands emerged in the genotypes White (80), Titicaca (42), Rainbow (38), Cherry Vanilla (37), Moqu Arrochilla (17), and China (13). Concurrently, previously existing bands were lost in China (54), Rainbow (35), Cherry Vanilla (27), Titicaca (20), Moqu Arrochilla (12), and White (8). These polymorphic

Genotype	Source	Properties	Stress-related Traits
Titicaca	Denmark	Early variety, whitish-yellow seed color	High drought and salinity tolerance; performs reliably in temperate to semi-arid conditions[48]
Rainbow	USA	Mid-late maturation variety, white seed color	Moderate drought tolerance with effective stomatal regulation under water deficit[49]
Moqu Arrochilla	Peru	Early variety, whitish seed color	Andean landrace highly adapted to arid conditions; shows deep root development and strong drought resilience[24]
Cherry Vanilla	USA	Mid-late maturation variety, white seed color	It is a drought-tolerant sea-level variety; although it exhibited some yield loss under the prolonged dry summer conditions of Pullman, WA, it demonstrated relatively good tolerance compared to other sea-level genotypes[24]
China	China	Mid-late maturation population, brown seed color	Originally from humid temperate regions; Chinese experiments using deficit irrigation show quinoa's drought resistance, though China genotype performs relatively weaker in arid conditions[50]
White	Peru	Late population, white seed color	Adapted to harsh Andean climates; known for strong drought resistance and effective performance in stress-prone soils[51]

Table 1. Quinoa genotypes used in the research and some of their properties.

iPBS Primers	Sequence (5'-3')	Tm (°C)	CG[1] (%)	Optimal Annealing Ta (°C)
2078	GCGGAGTCGCCA	54.2	75.0	62.8
2079	AGGTGGGCGCCA	56.6	75.0	65.2
2221	ACCTAGCTCACGATGCCA	58.0	55.6	56.9
2276	ACCTCTGATACCA	42.7	46.2	51.7
2298	AGAAGAGCTCTGATACCA	51.6	44.4	60.0
2377	ACGAAGGGACCA	47.2	58.3	53.0
2380	CAACCTGATCCA	41.4	50.0	50.5
2381	GTCCATCTTCCA	40.9	50.0	50.0
2384	GTAATGGGTCCA	40.9	50.0	50.0
2391	ATCTGTCAGCCA	43.6	50.0	52.6

Table 2. iPBS-retrotransposons primer names, sequence, the melting temperature (Tm), CG content (%) and annealing temperature used in this study. [1]CG: percentage of cytosine (C) and guanine (G) in the primary sequence, respectively.

changes, comprising both band gains and losses, reflect differential genomic responses to water stress. The extent of polymorphism varied across both genotypes and irrigation levels, indicating genotype-dependent genomic plasticity (Table 2).

The highest polymorphism rate (65.0%) was detected in the White genotype under 50% field capacity, whereas the lowest polymorphism (15.4%) was recorded in Moqu Arrochilla at 5% field capacity. In addition to polymorphism rates, changes in iPBS profiles were quantified using Genomic Template Stability (GTS) percentages, which serve as indicators of genomic stability in response to stress. GTS values were calculated for all 10 primers used in the study and are presented in Table 3. The response varied among genotypes: the highest GTS value (84.6%) was found in Moqu Arrochilla under the 5% FC treatment, while the lowest GTS (35.0%) was observed in White at 50% FC.

CRED-iPBS analysis The CRED-iPBS analysis was performed using ten primers (Table 2) to detect cytosine methylation alterations in genomic DNA. Genomic DNA was digested with MspI and HpaII, and polymorphism percentages were calculated based on changes relative to the control (undigested) DNA band profiles. The level of irrigation appeared to influence whether methylation changes represented hypermethylation or hypomethylation. In the MspI control group, the number of bands varied across genotypes: Moqu Arrochilla (63), China (70), Titicaca (72), White (74), Rainbow (75), and Cherry Vanilla (80). Compared to the controls, the number of newly formed bands in the MspI-digested experimental groups were as follows: China (58), Titicaca (51), Rainbow (46), White (46), Moqu Arrochilla (40), and Cherry Vanilla (30). Simultaneously, the number of lost bands was: White (57), Rainbow (53), Cherry Vanilla (51), Titicaca (46), Moqu Arrochilla (42), and China (35). The White genotype exhibited the greatest total number of altered bands (103), while Cherry Vanilla showed the lowest (81). The highest MspI polymorphism rate (42.9%) was observed in the China genotype under 50% field capacity, whereas the lowest (20.8%) was recorded in the Titicaca genotype under 5% field capacity.

The CRED-iPBS analysis using HpaII revealed varying band numbers across quinoa genotypes in the control groups: Moqu Arrochilla (59), Cherry Vanilla (69), Rainbow (71), White (72), Titicaca (72), and China (76). When experimental groups were compared to their respective controls, the number of newly formed bands was as follows: White (38), Moqu Arrochilla (37), Titicaca (32), Cherry Vanilla (31), China (30), and Rainbow (24). The corresponding number of lost bands was: Titicaca (65), China (61), White (59), Rainbow (53), Cherry Vanilla (46), and Moqu Arrochilla (42). The genotypes White and Titicaca exhibited the highest total number of altered bands (97 each), whereas Cherry Vanilla and Rainbow had the lowest (77 each). The highest polymorphism level for HpaII (39.0%) was recorded in Moqu Arrochilla at 5% field capacity, while the lowest (21.1%) was found in China at 25% field capacity (Table 4).

Primers	±[1]	Control[2] (100% FC)	Titicaca genotype			
			Irrigation levels (Field Capacity; FC)			
			5% (FC)	10% (FC)	25% (FC)	50% (FC)
iPBS 2078	+	8	1027	–	–	1016
	–		–	–	522; 621	–
iPBS 2079	+	7	–	1068; 9128; 680	1063	1057; 923; 704; 562
	–		775; 527; 486	–	–	–
iPBS 2221	+	9	–	–	–	–
	–		610; 390	610; 390	610; 390	610; 390
iPBS 2276	+	4	–	–	–	–
	–		–	–	791; 694	791; 694
iPBS 2298	+	2	–	500	460	–
	–		–	–	–	400
iPBS 2377	+	5	548	545	–	–
	–		–	–	–	–
iPBS 2380	+	1	609	895; 600	–	900; 619; 509
	–		–	–	–	–
iPBS 2381	+	7	–	–	–	1180
	–		–	–	862	862
iPBS 2384	+	3	685; 514	720; 583; 524	720; 588	678; 520; 470
	–		–	–	–	–
iPBS 2391	+	8	907; 830; 773	915; 830	919; 827; 614	903; 821; 769
	–		–1	–	–	–
Total band		54	13	14	14	21
Polymorphism (%)			24.1	25.9	25.9	38.9
GTS value			75.6	74.1	74.1	61.6
Primers	±[1]	Control[2] (100% FC)	Rainbow genotype			
			Irrigation levels (Field Capacity; FC)			
			5% (FC)	10% (FC)	25% (FC)	50% (FC)
iPBS 2078	+	8	1100; 1033; 981; 420	1122; 1072	1105; 1077; 420	1100; 1016; 413
	–		714; 646	714; 646	714; 646	714; 646
iPBS 2079	+	7	–	310	332	250
	–		–	700; 538; 464	700	768; 700; 538; 512; 494
iPBS 2221	+	4	–	717; 400	681; 537; 380	859; 668; 539; 390
	–		781; 514	–	–	–
iPBS 2276	+		–	–	–	–
	–		–	–	–	–
iPBS 2298	+	3	–	–	1083; 913	–
	–		–	495	–	495
iPBS 2377	+	6	–	–	–	–
	–		–	–	751; 527	–
iPBS 2380	+	2	–	–	–	–
	–		–	619	–	619
iPBS 2381	+	7	1100	–	–	978
	–		812; 518	848	848	812
iPBS 2384	+	1	–	1145; 907	1200; 960	1154; 907
	–		–	–	–	–
iPBS 2391	+	8	–	649	816	809; 637
	–		941; 691; 279	941	–	941
Total band		50	14	17	18	24
Polymorphism (%)			28.0	34.0	36.0	48.0
GTS value			72.0	66.0	64.0	52.0
Primer	±[1]	Control[2] (100% FC)	Moqu Arrochilla genotype			
			Irrigation levels (Field Capacity; FC)			
			5% (FC)	10% (FC)	25% (FC)	50% (FC)
Continued						

Primer	±[1]	Control[2] (100% FC)	Moqu Arrochilla genotype			
			Irrigation levels (Field Capacity; FC)			
			5% (FC)	10% (FC)	25% (FC)	50% (FC)
iPBS 2078	+	1	–	–	480	517; 480
	–		–	–	–	–
iPBS 2079	+	3	–	–	521; 295	–
	–		–	–		–
iPBS 2221	+	2	–	–	–	743; 554
	–		–	–	–	–
iPBS 2276	+	3	–	–	677	345
	–		566	–	–	–
iPBS 2298	+	2	–	–	–	275
	–		355	–	–	–
iPBS 2377	+	4	–	435	–	–
	–		–	–	563	856
iPBS 2380	+	2	–	–	–	344
	–		254	–	–	–
iPBS 2381	+	5	–	–	473	473
	–		300	741; 589; 378; 300	300	300
iPBS 2384	+	1	–	–	1145	–
	–		–	–	–	–
iPBS 2391	+	3	–	–	–	963; 608
	–		–	–	–	–
Total band		26	4	5	8	12
Polymorphism (%)			15.4	19.2	30.8	46.2
GTS value			84.6	80.2	69.2	53.8

Primers	±[1]	Control[2] (100% FC)	Cherry Vanilla genotype			
			Irrigation levels (Field Capacity; FC)			
			5% (FC)	10% (FC)	25% (FC)	50% (FC)
iPBS 2078	+	10	–	525	527	1211; 1038
	–		850; 475; 423	1127; 850; 452	850; 423	850
iPBS 2079	+	7	–	–	584	1042; 928; 680
	–		–	–	772; 419	263
iPBS 2221	+	5	641	–	661; 600	–
	–		–	520; 380	–	380
iPBS 2276	+	4	–	–	–	–
	–		427	–	–	–
iPBS 2298	+	2	–	–	–	807
	–		–	–	–	–
iPBS 2377	+	3	–	542	–	886; 539; 527
	–		–	–	–	–
iPBS 2380	+	2	–	–	749; 634	884; 763
	–		–	595	–	–
iPBS 2381	+	7	–	–	–	1180
	–		544	908; 842	908; 842; 544	544
iPBS 2384	+	3	1209	1200; 700; 583	1209; 726; 538	588; 530
	–		990	–	808	–
iPBS 2391	+	7	830; 736; 635	817; 630	–	828; 524
	–		305	–	–	–
Total band		50	12	15	17	20
Polymorphism (%)			24.0	30.0	34.0	40.0
GTS value			76.0	70.0	66.0	60.0

Primers	±[1]	Control[2] (100% FC)	China genotype			
			Irrigation levels (Field Capacity; FC)			
			5% (FC)	10% (FC)	25% (FC)	50% (FC)
iPBS 2078	+	12	–	–	–	–
	–		417; 392	659; 392	1005; 770; 659; 392	659; 392
iPBS 2079	+	7	–	–	–	–
	–		359	359	359	359
iPBS 2221	+	11	–	–	–	–
	–		834; 610; 438	875; 438	875; 610; 438; 375	834; 438; 375
iPBS 2276	+	4	–	–	–	–
	–		–	–	–	–
iPBS 2298	+	3	–	387	391	1100; 928
	–		–	–	–	–
iPBS 2377	+	5	–	523	523	527
	–		–	–	–	–
iPBS 2380	+	7	–	–	–	–
	–		880; 745; 493; 460; 418	880; 745; 591; 493; 460; 418	493; 460; 418	880; 745; 493; 460; 418
iPBS 2381	+	6	534	–	1080; 842	–
	–		–	–	–	–
iPBS 2384	+	3	–	–	–	–
	–		–	–	–	–
iPBS 2391	+	11	907	610	919	–
	–		792	792	792; 273	937; 691; 637; 561; 273
Total band		69	14	15	19	19
Polymorphism (%)			20.3	21.7	27.5	27.5
GTS value			79.7	78.3	72.5	72.5
Primers	±[1]	Control[2] (100% FC)	White genotype			
			Irrigation levels (Field Capacity; FC)			
			5% (FC)	10% (FC)	25% (FC)	50% (FC)
iPBS 2078	+	6	1133; 1016	1100; 1055; 993	1116; 1066; 1016	1122; 1016
	–		–	–	–	–
iPBS 2079	+	7	436	436	443	1089; 928
	–		600	600	600	–
iPBS 2221	+	2	717; 635; 569; 520	711; 514	869; 681; 600; 512; 404	859; 711; 648; 522
	–		–	–	–	–
iPBS 2276	+	2	782; 684	–	721	–
	–		–	–	–	–
iPBS 2298	+	2	525	–	532; 474	1016; 791; 491
	–		–	–	–	–
iPBS 2377	+	5	–	545	–	552
	–		–	–	–	–
iPBS 2380	+	2	771	763	–	880; 771; 493
	–		–	–	–	–
iPBS 2381	+	7	1100	957; 345	–	978
	–		842	1180	1180; 842	1180
iPBS 2384	+	3	–	706; 574; 525	706; 524; 191	532
	–		–	–	–	–

Continued

Primers	±[1]	Control[2] (100% FC)	White genotype			
			Irrigation levels (Field Capacity; FC)			
			5% (FC)	10% (FC)	25% (FC)	50% (FC)
iPBS 2391	+	4	932; 564; 273	928; 809; 769; 691; 561; 279	928; 843; 691; 561; 482; 292	900; 800; 709; 665; 626; 551; 521; 278
	–		–	–	–	–
Total band		40	17	21	24	26
Polymorphism (%)			42.5	52.5	60.0	65.0
GTS value			57.5	47.5	40.0	35.0

Table 3. The number of bands in control and disappearance (–), and/or appearance (+) of DNA bands with molecular sizes (base pair, bp), total band, polymorphism, and the average GTS value for all the primers of five Irrigation of field capacity (FC) treated quinoa genotypes. [1] + , – and [2]: appearance of a new band, disappearance of a normal band, and without drought stress, respectively.

Discussion

Quinoa stands out as a model crop in global agriculture due to its exceptional tolerance to abiotic stresses. Its high nutritional value rich in protein, essential amino acids, and micronutrients makes it a valuable food source in efforts to combat global hunger. Numerous studies have highlighted quinoa's efficient water use, high salinity tolerance, and adaptability to harsh environments, contributing to a growing global interest in its cultivation[25]. As a climate-resilient crop with notable resistance to salt, cold, and drought stress[26], quinoa offers promising solutions for sustainable food production, especially under low-input agricultural systems. The worldwide decline in crop productivity is largely driven by limited irrigation resources and increasing soil salinity[27]. Quinoa's ability to thrive under such conditions not only addresses food security concerns but also supports farmers in water-scarce regions and contributes to soil rehabilitation in saline lands[28].

Plants respond to drought stress through a series of morphological, physiological, biochemical, and molecular changes[29]. While previous studies have largely emphasized plant responses to acute water stress at specific developmental stages[30], little is known about quinoa's molecular response to prolonged drought across its entire growth cycle. The present study addresses this gap by evaluating DNA methylation patterns and genomic stability in six quinoa genotypes exposed to varying irrigation regimes using iPBS and CRED-iPBS markers. These methods provide a comprehensive assessment of both genetic alterations and cytosine methylation under drought stress.

The analysis of iPBS profiles revealed genotype-specific polymorphic patterns. The White genotype showed the greatest number of newly formed bands (80) and the fewest lost bands (8), indicating a dynamic genomic response to water limitation. In contrast, the China genotype displayed the fewest new bands (13) and the highest number of lost bands (54), suggesting limited adaptability at the genomic level. The GTS values further supported this observation, with the highest value (84.6%) recorded in Moqu Arrochilla at 5% field capacity and the lowest (35.0%) in White at 50% field capacity. These results highlight that drought stress induces substantial genomic rearrangements in quinoa, possibly due to DNA damage, activation of retrotransposons, or point mutations affecting oligonucleotide priming sites. Such modifications can alter the accessibility of priming regions, leading to the observed polymorphisms[31,32].

The findings of this study's CRED-iPBS analysis indicate that significant differences in the DNA band profiles of various quinoa genotypes, with variations observed across different irrigation levels of field capacity. The China genotype, irrigated at 50% field capacity, exhibited the highest polymorphism value for MspI (42.9%), while the experimental group of the Titicaca genotype, irrigated at 5% field capacity, showed the lowest value (20.8%). Similarly, Moqu Arrochilla genotype, irrigated at 5% field capacity, demonstrated the highest polymorphism value for HpaII (39.0%), whereas the China genotype, irrigated at 25% field capacity, displayed the lowest value (21.1%). While quinoa demonstrates remarkable resistance to stress, the precise mechanisms underlying this effect remain poorly understood. Research has shown that plants respond to biotic and abiotic stress through various mechanisms, including molecular, physiological, and biochemical pathways, which regulate gene expression[33]. Several studies have also demonstrated that water deficit stress can induce changes in DNA methylation in plants[34]. However, the exact relationship between DNA methylation and the water deficit tolerance mechanism in quinoa remains uncertain. Although the CRED-iPBS technique effectively identifies relative variations in cytosine methylation across different treatment conditions, it does not provide site-specific resolution. Therefore, future studies employing high-resolution approaches, such as bisulfite sequencing or methylation-specific PCR (MSP), are recommended to validate and localize these epigenetic changes more precisely. Plants and animals both undergo DNA methylation at specific positions, such as the cytosine of the CG dinucleotide, CNG, and CNN sequences[35]. By altering the stability and positioning of nucleosomes, the basic units of chromatin, this hereditary epigenetic mark regulates gene expression and chromatin structure[36]. Consequently, it affects the accessibility of DNA to regulatory proteins or protein complexes involved in DNA replication, repair, and transcription. Thus, DNA methylation influences responses to stress, flowering, and gene development in plants[37].

Approximately 20–30% of cytosines in the nuclear genome of plants are methylated, and methylation levels can vary significantly across tissues, organs, and developmental stages[38]. Alterations in DNA methylation may result in abnormal plant phenotypes, such as stunted growth, leaf clustering, or delayed flowering, which can be

Primers	M/H[1]	±[2]	Control[3] (100% FC)	Titicaca genotype			
				Irrigation levels (Field Capacity; FC)			
				5% (FC)	10% (FC)	25% (FC)	50% (FC)
iPBS 2078	M	+	8	387; 300; 253	421; 391; 364; 341; 150	430; 375	430; 379; 360; 352; 311; 292
		–		–	–	531	531; 495
	H	+	10	450; 379; 341; 296; 150	387; 364; 322	440; 383; 367; 352	430; 375; 341; 322
		–		724; 657	724; 600	724; 657; 600; 543	724; 657
iPBS 2079	M	+	6	728	–	–	–
		–		338	338	338	338
	H	+	4	–	–	–	839
		–		–	–	409	–
iPBS 2221	M	+	10	–	673	–	–
		–		–	847;330	847; 808; 722; 500; 330	847; 500
	H	+	8	–	–	–	–
		–		658; 500; 330	910; 847; 330	500	330
iPBS 2276	M	+	6	–	432; 258; 234	237	432; 231
		–		–	–	–	–
	H	+	7	–	–	–	220
		–		439	439	439	439
iPBS 2298	M	+	9	239	249	585; 249	300; 249
		–		–	622	–	622; 518
	H	+	10	300	410	380	433
		–		613; 592	613; 592; 527	592	613; 592
iPBS 2377	M	+	4	525; 393; 309	–	525; 509; 388; 328	635; 528; 333; 304
		–		–	437; 196	–	–
	H	+	8	–	254	343	–
		–		525; 500; 449; 424; 282	525; 500; 449	525; 449; 424	500; 449; 258
iPBS 2380	M	+	8	300	–	300	295
		–		–	122	224; 152; 122	–
	H	+	8	185	185; 160	–	–
		–		295	–	295	–
iPBS 2381	M	+	8	292	292	270	368
		–		560; 435	560	560	–
	H	+	9	–	–	–	–
		–		639; 622; 600; 443	639; 622; 333	639; 622; 333	622; 600
iPBS 2384	M	+	5	400	400	394	–
		–		319	319; 288	319	319; 288; 244; 225
	H	+	1	250	–	394; 225	307
		–		–	–	–	–
iPBS 2391	M	+	8	–	–	–	–
		–		–	550; 238; 205; 177	550; 238; 205	238; 205
	H	+	5	195	–	–	–
		–		–	379; 307	379	445; 379; 341
Polymorphism %	M			20.8	36.1	37.5	41.4
	H			38.6	34.3	34.3	30.0
Primers	M/H[1]	±[2]	Control[3] (100% FC)	Rainbow genotype			
				Irrigation levels (Field Capacity; FC)			
				5% (FC)	10% (FC)	25% (FC)	50% (FC)
iPBS 2078	M	+	10	777; 754; 741;649;393; 322	665; 393; 231; 206	680;206	777; 754; 737; 378; 300; 202
		–		–	423	423	–
	H	+	11	–	770	791; 759; 728	777; 754; 737; 307
		–		624	562; 254; 209	–	418
iPBS 2079	M	+	7	–	–	–	–
		–		858; 728	858; 728	858; 728	789
	H	+	6	–	–	–	–
		–		858; 800	–	858; 800	–

Continued

Primers	M/H[1]	±[2]	Control[3] (100% FC)	Rainbow genotype			
				Irrigation levels (Field Capacity; FC)			
				5% (FC)	10% (FC)	25% (FC)	50% (FC)
iPBS 2221	M	+	9	847	867; 800	791	1134
		–		–	980; 711; 572	980; 432	711; 658
	H	+	8	330	–	–	732
		–		1200; 867; 600	1200; 379	1200; 791	1200
iPBS 2276	M	+	7	246	246	246	252
		–		–	–	439	513; 439; 150
	H	+	8	–	–	–	–
		–		516; 483; 277; 252; 150	483; 277; 252	483; 252	483; 252
iPBS 2298	M	+	10	235; 216	–	–	288;230; 196
		–		–	527	596; 527; 433	433; 145
	H	+	10	–	–	–	–
		–		433; 265	265	–	–
iPBS 2377	M	+	5	500; 382; 228	218	218	500; 443; 393
		–		–	–	513; 349	232
	H	+	7	–	254	–	–
		–		–	430	513; 430; 370; 235	489; 430
iPBS 2380	M	+	8	–	277	492	281
		–		224	191	249; 150	191
	H	+	6	295; 145	300; 163	145; 163	295; 160;145
		–		–	–	–	–
iPBS 2381	M	+	9	545	–	–	–
		–		–	588; 300	639; 622; 588; 443; 328; 300	622; 588
	H	+	5	633; 609; 300	–	–	–
		–		–	–	463	463
iPBS 2384	M	+	4	–	262	–	–
		–		384; 236	384	–	384
	H	+	4	–	288	–	–
		–		379	379	379	379; 216
iPBS 2391	M	+	6	–	–	–	226
		–		–	550; 292	550; 379; 177	–
	H	+	6	–	–	–	–
		–		558; 379; 307	558; 379; 181	–	558
Polymorphism %	M			25.3	30.7	37.3	38.7
	H			32.4	26.8	23.9	25.4
Primers	M/H[1]	±[2]	Control[3] (100% FC)	Moqu Arrochilla genotype			
				Irrigation levels (Field Capacity; FC)			
				5% (FC)	10% (FC)	25% (FC)	50% (FC)
iPBS 2078	M	+	14	–	–	–	–
		–		–	700; 680; 241	700; 680; 593; 538; 492; 241	593; 538; 379
	H	+	11	606	–	700; 600; 333	600
		–		356; 311	400	261	261
iPBS 2079	M	+	2	–	–	–	–
		–		–	–	–	–
	H	+	2	–	–	–	–
		–		–	–	–	–
iPBS 2221	M	+	7	–	1018; 831	811; 616; 566	855; 663; 263
		–		421; 351	421	421; 351	421
	H	+	7	–	250	300	–
		–		876; 608; 566	608; 466	–	–
iPBS 2276	M	+	4	–	–	–	262; 157
		–		–	–	–	–
	H	+	4	–	157	–	–
		–		–	–	265	237

Continued

Primers	M/H[1]	±[2]	Control[3] (100% FC)	Moqu Arrochilla genotype Irrigation levels (Field Capacity; FC) 5% (FC)	10% (FC)	25% (FC)	50% (FC)
iPBS 2298	M	+	7	600; 564; 500; 313	527; 341; 300	334	500; 378; 319
		–		234; 139	234	234	–
	H	+	13	–	–	–	–
		–		606; 569; 164; 139	606; 569; 500; 388; 313; 204; 164	606; 569; 500; 204; 164	606; 569; 500; 388; 204; 164; 139
iPBS 2377	M	+	489 309	–	489	–	–
		–		467; 393; 309; 262	309	612; 309; 262	467; 393; 343; 309; 262
	H	+	608; 258; 200 –	–	608; 258; 200	348; 161	343; 163
		–		–	–	–	–
iPBS 2380	M	+	4	240; 211; 166	207	272; 227; 211	451; 400; 227; 157
		–		–	–	–	–
	H	+	6	–	–	295	295; 172
		–		–	–	–	–
iPBS 2381	M	+	7	372	–	–	–
		–		–	–	–	–
	H	+	7	286	–	–	–
		–		621; 607	621; 607	–	621; 607
iPBS 2384	M	+	1	236; 215	262; 240; 123	–	245
		–		–	–	–	–
	H	+	1	232; 211; 135	215	–	219; 141
		–		–	–	–	–
iPBS 2391	M	+	10	–	–	–	–
		–		237; 217	451; 237; 217	–	451; 237
	H	+	4	281; 264; 217; 165	281; 258; 162	281; 258; 162	287; 165
		–		–	–	–	536
Polymorphism %	M			28.6	31.7	33.3	36.5
	H			39.0	33.9	28.8	32.2
Primers	M/H[1]	±[2]	Control[3] (100% FC)	Cherry Vanilla genotype Irrigation levels (Field Capacity; FC) 5% (FC)	10% (FC)	25% (FC)	50% (FC)
iPBS 2078	M	+	12	593	–	661; 595; 461; 445; 408; 356	600
		–		371	695; 680; 296	–	371; 273
	H	+	12	695	450	700; 421	473
		–		232	–	379; 232; 184	585; 379; 303; 184
iPBS 2079	M	+	5	–	–	669	308
		–		–	–	–	800; 439
	H	+	4	790	800	–	–
		–		–	–	–	–
iPBS 2221	M	+	9	–	279	–	–
		–		1166; 910; 857; 523; 488; 310	910; 523	1166; 523; 310	1166
	H	+	8	1104	732	1000; 783; 658; 559	–
		–		1517; 614; 379	330	1517; 379	1517; 300
iPBS 2276	M	+	5	226	–	–	–
		–		–	–	177	–
	H	+	5	–	–	–	–
		–		272; 246	181	246	246; 181
iPBS 2298	M	+	10	589; 451	424	594; 579; 368	368
		–		275	275	275	–
	H	+	10	579	–	579; 275	287
		–		–	600; 510	136	–

Continued

Primers	M/H[1]	±[2]	Control[3] (100% FC)	Cherry Vanilla genotype Irrigation levels (Field Capacity; FC) 5% (FC)	10% (FC)	25% (FC)	50% (FC)
iPBS 2377	M	+	11	265	254	–	243
		–		500; 473; 313	500; 473; 443	473; 338; 313	500; 473; 313
	H	+	10	–	–	–	500
		–		400; 286; 254	365; 323; 286; 254; 232; 200	629; 323; 286; 254	286; 254
iPBS 2380	M	+	8	–	–	400; 369	–
		–		–	290; 185	–	290; 185
	H	+	6	200	202	–	–
		–		–	–	–	–
iPBS 2381	M	+	8	–	590; 450	–	–
		–		353	–	353	–
	H	+	6	614	–	607; 347; 319	614
		–		–	–	–	–
iPBS 2384	M	+	3	281	369; 202	–	281
		–		–	–	225	–
	H	+	2	–	271	–	281
		–		–	–	247	–
iPBS 2391	M	+	9	–	–	–	–
		–		172	172	172	300; 269; 222; 191; 172
	H	+	6	536; 264	536	–	536
		–		–	293; 227; 172	356; 326	–
Polymorphism %	M			23.8	23.8	27.5	29.00
	H			24.6	27.5	36.2	23.2
Primers	M/H[1]	±[2]	Control[3] (100% FC)	China genotype Irrigation levels (Field Capacity; FC) 5% (FC)	10% (FC)	25% (FC)	50% (FC)
iPBS 2078	M	+	11	543	338	600; 479	784; 759; 715; 672; 338
		–		231	231	231; 209	254; 231; 209
	H	+	14	777; 759	–	–	791; 770; 715
		–		624; 433; 400; 354; 330	672; 624; 433; 330; 259; 211	433; 400; 330	624; 549; 454; 330; 211
iPBS 2079	M	+	4	810	867; 789; 728	884; 810	875; 810
		–		–	–	–	–
	H	+	4	–	867; 789	–	810
		–		–	–	–	–
iPBS 2221	M	+	6	1074; 878; 808; 673	–	1104; 878; 837	963; 837; 791; 687
		–		410	–	235	410
	H	+	11	1373	–	–	–
		–		800; 751; 732	857; 700; 300	700; 300; 235	857; 732; 235
iPBS 2276	M	+	7	–	–	452	220
		–		–	–	240	–
	H	+	5	273	273	273	273
		–		183	–	–	–
iPBS 2298	M	+	8	596; 518; 294	592; 518; 196	527; 421	622; 527; 380
		–		–	–	142	230
	H	+	10	–	622; 596	200	622; 306
		–		380	–	–	–
iPBS 2377	M	+	4	206	513; 495; 418; 370; 239; 212	–	513; 479; 232
		–		359; 328	–	359; 328	–
	H	+	5	–	513; 269	254	–
		–		461; 393	–	–	–

Continued

Primers	M/H[1]	±[2]	Control[3] (100% FC)	China genotype			
				Irrigation levels (Field Capacity; FC)			
				5% (FC)	10% (FC)	25% (FC)	50% (FC)
iPBS 2380	M	+	9	–	451	460	485
		–		–	272; 152; 145; 117	272; 145; 117	272
	H	+	8	158	166	163	168
		–		145; 119	145; 119	145; 119	145
iPBS 2381	M	+	10	–	–	–	–
		–		–	–	616; 575; 252	575; 300; 252
	H	+	9	–	–	–	–
		–		–	646; 609; 560; 463	–	300
iPBS 2384	M	+	5	374	384	374	–
		–		–	–	209	225; 209
	H	+	4	300	–	253	–
		–		127	271; 127	127	127
iPBS 2391	M	+	6	–	–	323	–
		–		292	–	–	–
	H	+	6	369	379	389	–
		–		277; 226	226	226	535; 277; 226
Polymorphism %	M			22.9	28.6	38.6	42.9
	H			31.6	35.5	21.1	29.0
Primers	M/H[1]	±[2]	Control[3] (100% FC)	Cherry Vanilla genotype			
				Irrigation levels (Field Capacity; FC)			
				5% (FC)	10% (FC)	25% (FC)	50% (FC)
iPBS 2078	M	+		593	–	661; 595; 461; 445; 408; 356	600
		–		371	695; 680; 296	–	371; 273
	H	+		695	450	700; 421	473
		–		232	–	379; 232; 184	585; 379; 303; 184
iPBS 2079	M	+		–	–	669	308
		–		–	–	–	800; 439
	H	+		790	800	–	–
		–		–	–	–	–
iPBS 2221	M	+		–	279	–	–
		–		1166; 910; 857; 523; 488; 310	910; 523	1166; 523; 310	1166
	H	+		1104	732	1000; 783; 658; 559	–
		–		1517; 614; 379	330	1517; 379	1517; 300
iPBS 2276	M	+		226	–	–	–
		–		–	–	177	–
	H	+		–	–	–	–
		–		272; 246	181	246	246; 181
iPBS 2298	M	+		589; 451	424	594; 579; 368	368
		–		275	275	275	–
	H	+		579	–	579; 275	287
		–		–	600; 510	136	–
iPBS 2377	M	+		265	254	–	243
		–		500; 473; 313	500; 473; 443	473; 338; 313	500; 473; 313
	H	+		–	–	–	500
		–		400; 286; 254	365; 323; 286; 254; 232; 200	629; 323; 286; 254	286; 254
iPBS 2380	M	+		–	–	400; 369	–
		–		–	290; 185	–	290; 185
	H	+		200	202	–	–
		–		–	–	–	–

Continued

Primers	M/H[1]	±[2]	Control[3] (100% FC)	Cherry Vanilla genotype Irrigation levels (Field Capacity; FC) 5% (FC)	10% (FC)	25% (FC)	50% (FC)
iPBS 2381	M	+		–	590; 450	–	–
		–		353	–	353	–
	H	+		614	–	607; 347; 319	614
		–		–	–	–	–
iPBS 2384	M	+		281	369; 202	–	281
		–		–	–	225	–
	H	+		–	271	–	281
		–		–	–	247	–
iPBS 2391	M	+		–	–	–	–
		–		172	172	172	300; 269; 222; 191; 172
	H	+		536; 264	536	–	536
		–		–	293; 227; 172	356; 326	–
Polymorphism %	M			23.8	23.8	27.5	29.0
	H			24.6	27.5	36.2	23.2
Primers	M/H[1]	±[2]	Control[3] (100% FC)	White genotype Irrigation levels (Field Capacity; FC) 5% (FC)	10% (FC)	25% (FC)	50% (FC)
iPBS 2078	M	+	9	341	367; 333	461; 364; 341; 329	344
		–		–	578; 514	–	–
	H	+	9	585	700; 680; 578; 430; 300	522; 408; 300	578
		–		–	–	–	–
iPBS 2079	M	+	5	–	–	730	439
		–		–	–	–	–
	H	+	4	810	819	809	800
		–		–	600	–	–
iPBS 2221	M	+	8	687	1200	1000; 722	1587; 1000
		–		867; 559;443	443	339	808; 443
	H	+	7	922	922	922	1552; 722
		–		400	–	–	687; 586; 330
iPBS 2276	M	+	6	–	–	–	–
		–		439; 176	439; 176	439	439
	H	+	8	–	–	–	–
		–		500; 419; 261; 223; 139	419; 261; 223; 139	419; 223; 139	419; 261; 223; 139
iPBS 2298	M	+	10	378; 123	121	412; 368; 121	–
		–		622	622	622; 249	622
	H	+	13	339	–	175	–
		–		600; 225; 212	622; 527; 276	622; 600; 527; 276; 225; 212	622; 433
iPBS 2377	M	+	10	–	228	542; 313	–
		–		635; 250; 196	509; 467	250	525; 509; 467; 393; 286
	H	+	6	–	–	640; 479; 291; 250	–
		–		509; 393	509; 393	–	509; 393; 225
iPBS 2380	M	+	9	434	–	–	–
		–		228; 182	228; 182	228; 182	228; 119
	H	+	8	–	349; 290	349	–
		–		122	245; 191; 122	224; 191; 122	191; 122
iPBS 2381	M	+	9	–	611	607	614
		–		529; 368	529; 318	529; 368	529; 368
	H	+	7	–	614	607	–
		–		–	–	–	–
iPBS 2384	M	+	4	–	231	233	–
		–		405; 307; 271	405	405; 307	405; 307
	H	+	3	–	–	285	–
		–		405; 253	405	405	405; 253

Continued

Primers	M/H[1]	±[2]	Control[3] (100% FC)	White genotype			
				Irrigation levels (Field Capacity; FC)			
				5% (FC)	10% (FC)	25% (FC)	50% (FC)
iPBS 2391	M	+	4	–	523; 364; 300	529; 306	723; 614; 570; 523; 500; 356; 275; 227; 172; 141
		–		433; 292	–	–	–
	H	+	7	–	–	–	623; 564; 500; 293; 242; 183; 130
		–		–	–	348; 227	–
Polymorphism %	M			31.1	31.1	36.5	40.5
	H			25.0	33.3	38.9	37.5

Table 4. The changes in methylation status (CRED–iPBS) of quinoa genotypes exposed to different Irrigation of field capacity (FC). [1–3] M—Msp I, H—Hpa II; (+) appearance of a new band, (–) disappearance of a normal band; and without drought stress, respectively.

heritable across generations, as observed in Arabidopsis thaliana with reduced methylation[39]. DNA methylation patterns are known to be dynamic and responsive to environmental stimuli, including salinity[40], drought[41], low temperature[42], heavy metal exposure[21], and pathogen attacks[43]. The modulation of cytosine methylation under such adverse conditions plays a critical regulatory role in stress response and adaptation[34]. However, few studies have specifically addressed how water deficit influences DNA methylation in quinoa.

Conclusions

This study represents the first comprehensive evaluation of drought-induced genomic instability and cytosine methylation changes in quinoa genotypes using iPBS and CRED-iPBS marker systems. The findings demonstrate that limited irrigation triggers genotype-specific alterations in DNA band profiles, retrotransposon activity, and methylation patterns. iPBS analysis revealed substantial polymorphism and variable GTS values, indicating genomic rearrangements linked to water stress, while CRED-iPBS results confirmed both hypermethylation and hypomethylation events under different irrigation levels. These observations suggest that epigenetic reprogramming contributes significantly to quinoa's adaptive response to drought. The combined use of iPBS and CRED-iPBS markers proved effective in detecting stress-responsive genomic and epigenetic variations, providing insights into the regulatory mechanisms underlying resilience. Future studies incorporating larger genotype panels, transcriptomic profiling, and high-resolution methylation techniques such as bisulfite sequencing are recommended to validate and expand these findings. Overall, this research underscores the importance of understanding methylation-mediated responses to drought and highlights quinoa's potential as a model species for studying epigenetic adaptation in climate-resilient crops.

Materials and methods

Plant material The present study was conducted as a pot experiment in the greenhouse facilities of the Faculty of Agriculture at Atatürk University. Six quinoa (*Chenopodium quinoa* Willd.) genotypes, sourced from diverse geographical origins, were selected for evaluation. Detailed information on the genetic materials and their distinguishing characteristics is provided in Table 1.

Plant growth conditions and application The experiment was conducted under controlled greenhouse conditions using a factorial arrangement based on a completely randomized design (CRD) with three replications. Ten seeds were initially sown per pot, and seedlings were thinned to three plants after emergence. Uniform irrigation was applied during the first two weeks to maintain field capacity across all treatments. From the third week onward, five irrigation levels 100% field capacity (FC, control), 50% FC (mild drought), 25% FC (moderate drought), 10% FC (severe drought), and 5% FC (extreme drought) were applied to simulate drought stress. The greenhouse was maintained at a day/night temperature regime of 25/15 ± 5 °C. Each pot was filled with 2 kg of loamy garden soil mixed with 10% decomposed farmyard manure. Soil analysis indicated high organic matter (4.7%), neutral pH (7.04), non-saline conditions (total salt: 0.032%), low lime content (0.72%), and sufficient available phosphorus (4.20 ppm). Pots were weighed daily at a fixed time, and the amount of water needed to restore field capacity was added accordingly. The experiment was concluded eight weeks after the application of drought treatments, after which shoot and root length, shoot and root dry weight, root-to-shoot ratio, and drought tolerance percentage were measured[24].

DNA isolation, iPBS-PCR and CRED-iPBS amplification Leaf tissues were harvested from young seedlings of quinoa genotypes grown under both control and drought stress conditions. Genomic DNA was extracted using the method described by Zeinalzadehtabrizi et al.[44] and stored at − 20 °C for downstream analysis. DNA concentration and quality were assessed using a spectrophotometer and by electrophoresis on a 0.8% agarose gel, respectively. A total of 20 iPBS primers were initially screened using the amplification protocol described by Kalendar et al. [45]. PCR reactions were prepared in a 20 µL volume containing 10× buffer, 2 mM $MgCl_2$, 0.25 mM of each dNTP, 2 µM (20 pmol) primer, 0.5 U Taq polymerase, and 1 µL of template DNA (50 ng μL^{-1}). The amplification conditions included an initial denaturation at 95 °C for 3 min; 38 cycles of 95 °C for 15 s, 51–56 °C for 60 s, and 72 °C for 60 s; followed by a final extension at 72 °C for 5 min. PCR products were separated on 1.5% agarose gels prepared with 1× sodium borate (SB) buffer and run at 100 V/cm for 120 min. Gels were stained

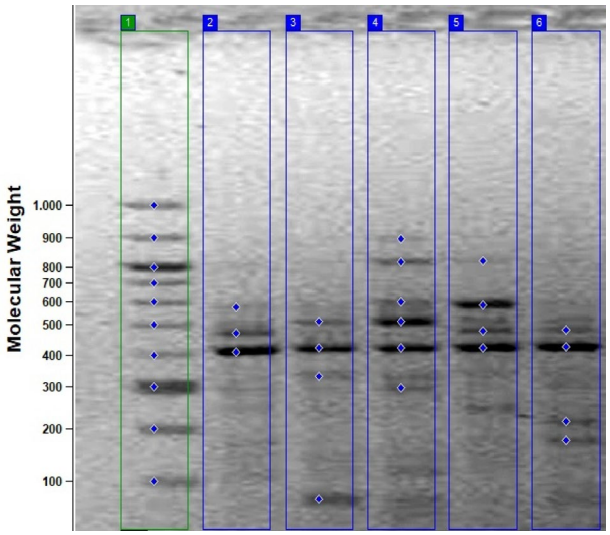

Fig. 1. iPBS banding profiles of iPBS-2078 marker across various drought stresstreatments in Titicaca genotype; 1: M 100–1000 bp DNA ladder; 2: control; 3: 5% FCtreatment; 4: 10% FC treatment; 5: 25% FC treatment; 6: 50% FC treatment.

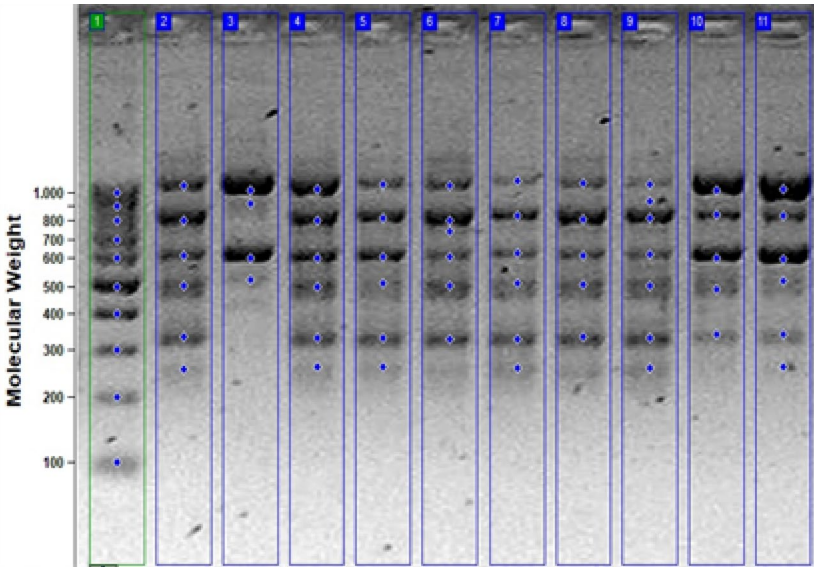

Fig. 2. CRED-iPBS profiles banding profiles of iPBS-2078 marker across various drought stress treatments in Titicaca genotype; 1: M, 100–1000 bp DNA ladder; 2: control *Hpa II*; 3: control *Msp I*; 4: 5% FC treatment *Hpa II*; 5: 5% FC treatment *Msp I*; 6: 10% FC treatment *Hpa II*; 7: 10% FC treatment *Msp I*; 8: 25% FC treatment *Hpa II*; 9: 25% FC treatment *Msp I*; 10: 50% FC treatment *Hpa II*; 11: 50% FC treatment *Msp I*.

with ethidium bromide (1.3 mM) and visualized under UV illumination. A 100 bp DNA ladder (Vivantis, NM2421) was used to estimate fragment sizes. Ten of the twenty iPBS primers yielded reproducible and scorable banding patterns across the six quinoa genotypes (Table 2). For CRED-iPBS analysis, 1000 ng of genomic DNA was digested with 1 U of either HpaII or MspI (Thermo Scientific) according to the manufacturer's instructions. The PCR conditions for CRED-iPBS were the same as those used for iPBS-PCR, except for the use of enzyme-digested DNA as the template. The CRED-iPBS amplification protocol consisted of an initial denaturation at 95 °C for 5 min; 40 cycles of 94 °C for 60 s, 51–56 °C for 60 s, and 72 °C for 120 s; and a final extension at 72 °C for 15 min. The resulting products were separated on 1.5% agarose gels in 1× SB buffer, stained with ethidium bromide (0.2 µg mL^{-1}), and visualized under UV light[21].

iPBS and CRED-iPBS analyses The banding patterns generated from iPBS and CRED-iPBS analyses were evaluated using TotalLab TL120 software (Nonlinear Dynamics Ltd) (Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 12). Genomic Template Stability (GTS%) was calculated using the formula: GTS = 1 – (a/n), where a is the average number of polymorphic bands in each treated sample and n is the total number of bands in the control group[46].

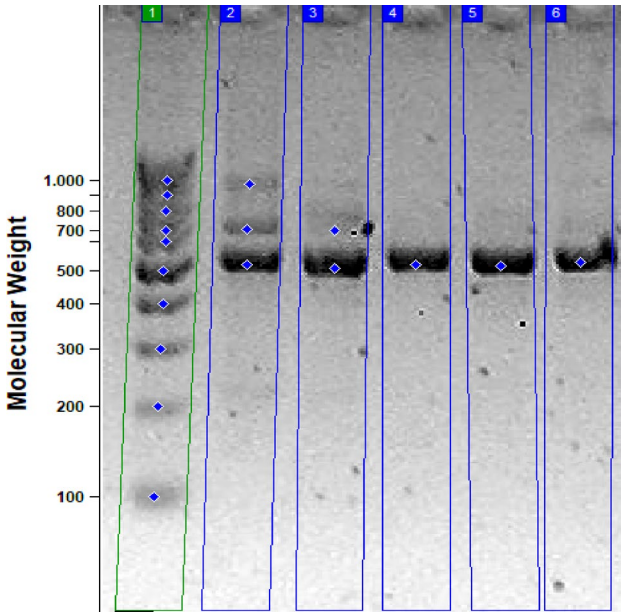

Fig. 3. iPBS banding profiles of iPBS-2077 marker across various drought stress treatments in Rainbow genotype; 1: M 100–1000 bp DNA ladder; 2: control; 3: 5% FC treatment; 4: 10% FC treatment; 5: 25% FC treatment; 6: 50% FC treatment.

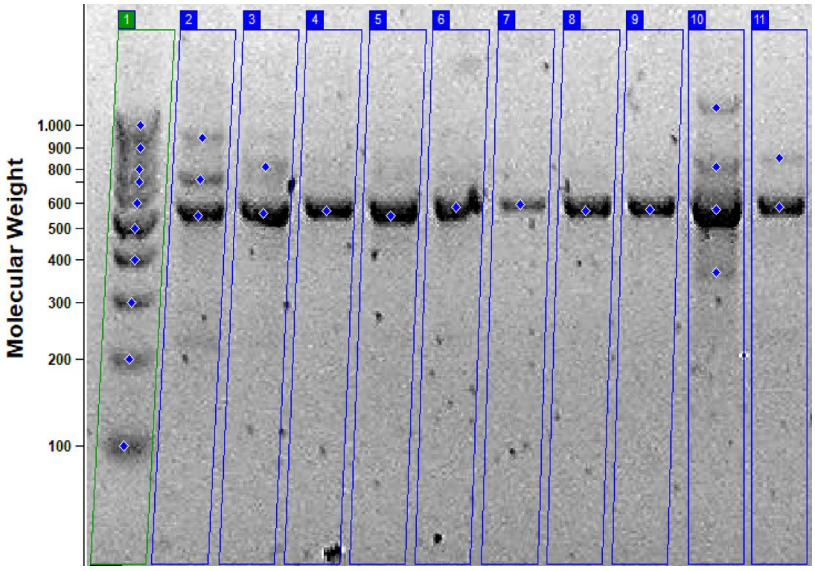

Fig. 4. CRED-iPBS profiles banding profiles of iPBS-2077 marker across various drought stress treatments in Rainbow genotype; 1: M, 100–1000 bp DNA ladder; 2: control *Hpa II*; 3: control *Msp I*; 4: 5% FC treatment *Hpa II*; 5: 5% FC treatment *Msp I*; 6: 10% FC treatment *Hpa II*; 7: 10% FC treatment *Msp I*; 8: 25% FC treatment *Hpa II*; 9: 25% FC treatment *Msp I*; 10: 50% FC treatment *Hpa II*; 11: 50% FC treatment *Msp I*.

Polymorphisms in iPBS profiles were identified based on the disappearance of existing bands and the appearance of novel bands relative to the control. The mean polymorphism rate for each treatment group was expressed as a percentage of the control (set at 100%). For the CRED-iPBS analysis, the average polymorphism percentage for each treatment concentration was calculated using the formula: $100 \times a/n$[47].

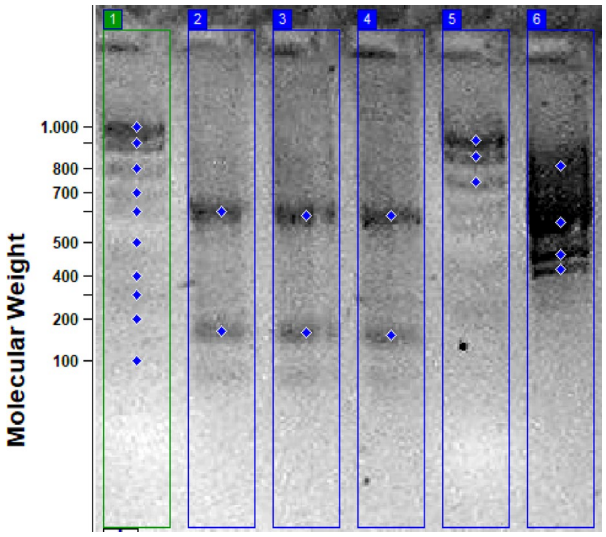

Fig. 5. iPBS banding profiles of iPBS-2381 marker across various drought stress treatments in Moqu Arrochilla genotype; 1: M 100–1000 bp DNA ladder; 2: control; 3: 5% FC treatment; 4: 10% FC treatment; 5: 25% FC treatment; 6: 50% FC treatment.

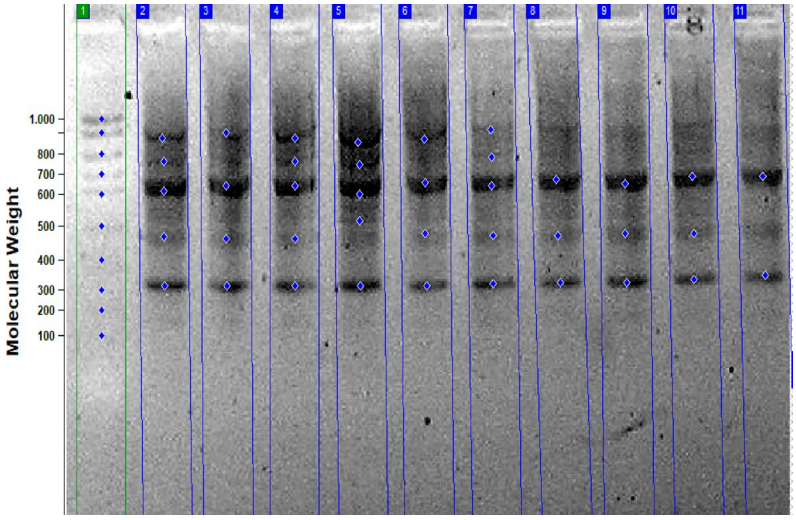

Fig. 6. CRED-iPBS profiles banding profiles of iPBS-2381 marker across various drought stress treatments in Moqu Arrochilla genotype; 1: M, 100–1000 bp DNA ladder; 2: control *Hpa II*; 3: control *Msp I*; 4: 5% FC treatment *Hpa II*; 5: 5% FC treatment *Msp I*; 6: 10% FC treatment *Hpa II*; 7: 10% FC treatment *Msp I*; 8: 25% FC treatment *Hpa II*; 9: 25% FC treatment *Msp I*; 10: 50% FC treatment *Hpa II*; 11: 50% FC treatment *Msp I*.

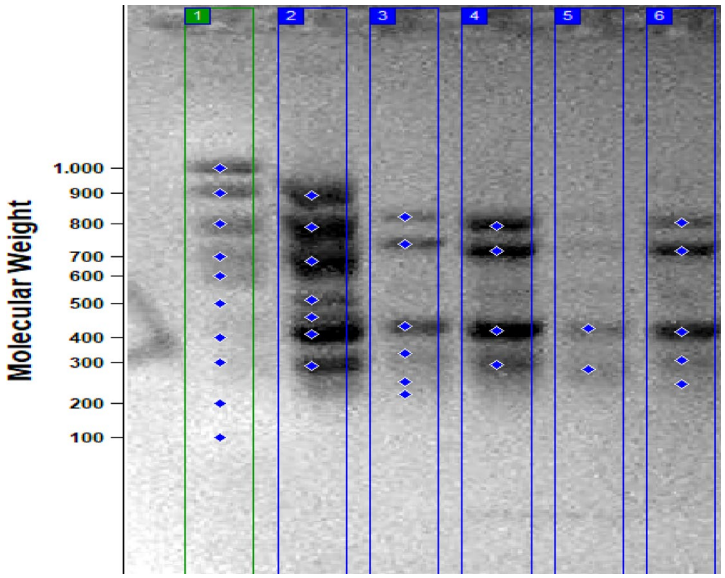

Fig. 7. iPBS banding profiles of iPBS-2389 marker across various drought stress treatments in Cherry Vanilla genotype; 1: M 100–1000 bp DNA ladder; 2: control; 3: 5% FC treatment; 4: 10% FC treatment; 5: 25% FC treatment; 6: 50% FC treatment.

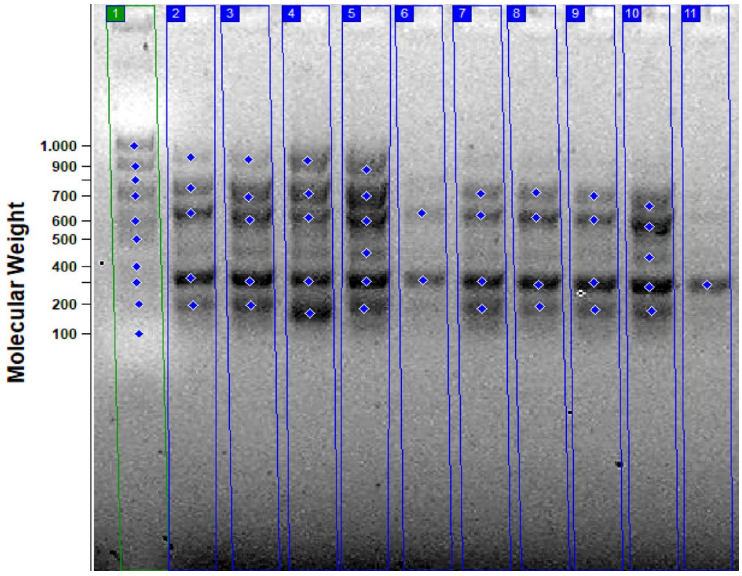

Fig. 8. CRED-iPBS profiles banding profiles of iPBS-2389 marker across various drought stress treatments in Cherry Vanilla genotype; 1: M, 100–1000 bp DNA ladder; 2: control *Hpa II*; 3: control *Msp I*; 4: 5% FC treatment *Hpa II*; 5: 5% FC treatment *Msp I*; 6: 10% FC treatment *Hpa II*; 7: 10% FC treatment *Msp I*; 8: 25% FC treatment *Hpa II*; 9: 25% FC treatment *Msp I*; 10: 50% FC treatment *Hpa II*; 11: 50% FC treatment *Msp I*.

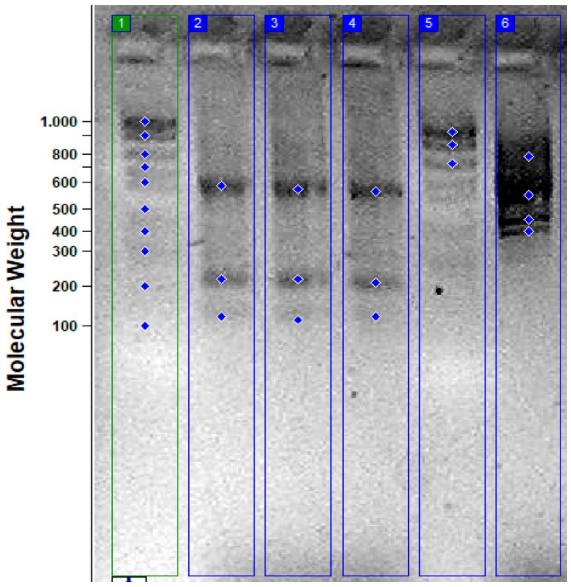

Fig. 9. iPBS banding profiles of iPBS-2390 marker across various drought stress treatments in China genotype; 1: M 100–1000 bp DNA ladder; 2: control; 3: 5% FC treatment; 4: 10% FC treatment; 5: 25% FC treatment; 6: 50% FC treatment.

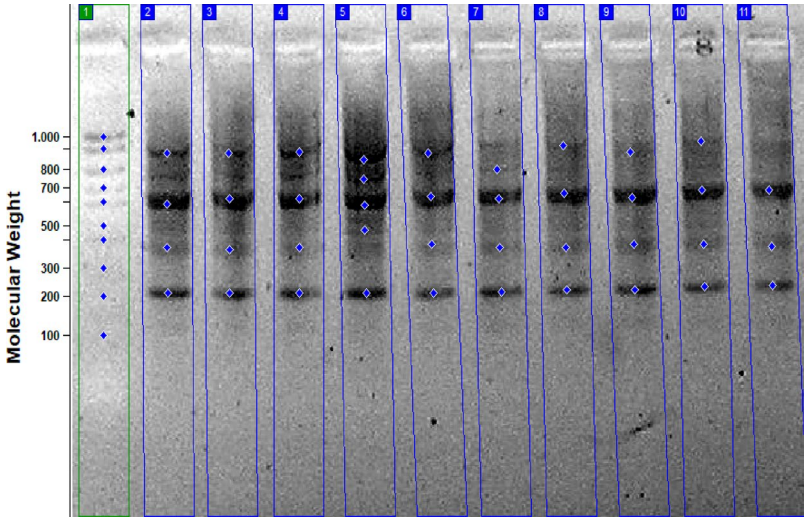

Fig. 10. CRED-iPBS profiles banding profiles of iPBS-2390 marker across various drought stress treatments in China genotype; 1: M, 100–1000 bp DNA ladder; 2: control *Hpa II*; 3: control *Msp I*; 4: 5% FC treatment *Hpa II*; 5: 5% FC treatment *Msp I*; 6: 10% FC treatment *Hpa II*; 7: 10% FC treatment *Msp I*; 8: 25% FC treatment *Hpa II*; 9: 25% FC treatment *Msp I*; 10: 50% FC treatment *Hpa II*; 11: 50% FC treatment *Msp I*.

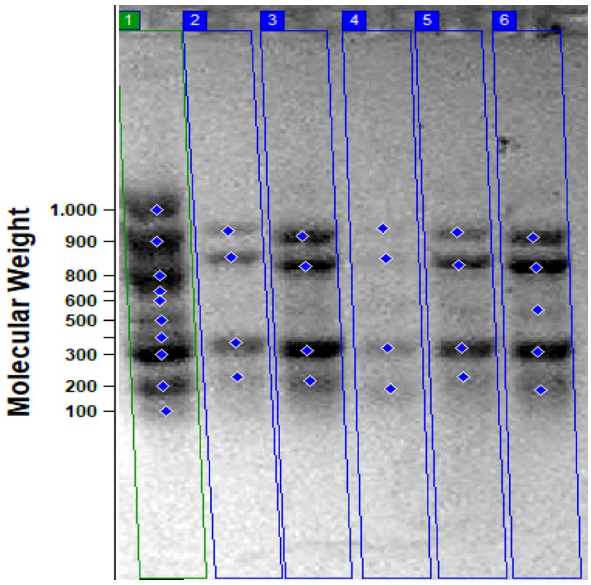

Fig. 11. iPBS banding profiles of iPBS-2391 marker across various drought stress treatments in White genotype; 1: M 100–1000 bp DNA ladder; 2: control; 3: 5% FC treatment; 4: 10% FC treatment; 5: 25% FC treatment; 6: 50% FC treatment.

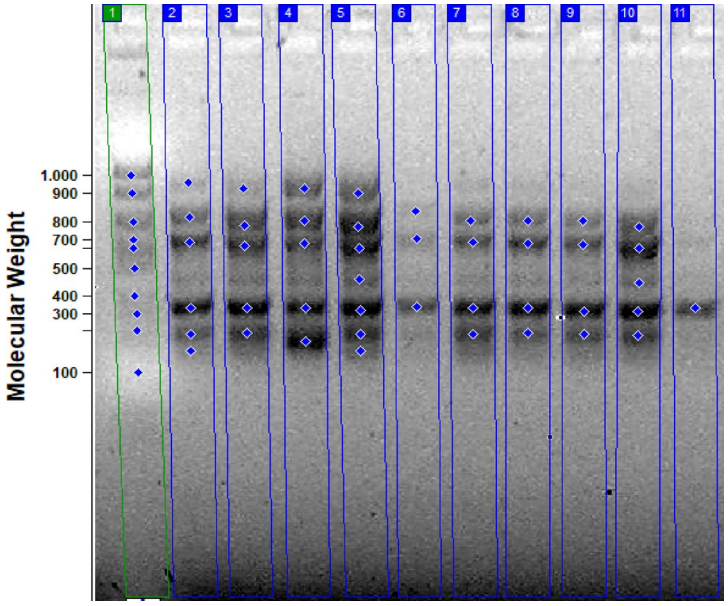

Fig. 12. CRED-iPBS profiles banding profiles of iPBS-2391 marker across various drought stress treatments in White genotype; 1: M, 100–1000 bp DNA ladder; 2: control *Hpa II*; 3: control *Msp I*; 4: 5% FC treatment *Hpa II*; 5: 5% FC treatment *Msp I*; 6: 10% FC treatment *Hpa II*; 7: 10% FC treatment *Msp I*; 8: 25% FC treatment *Hpa II*; 9: 25% FC treatment *Msp I*; 10: 50% FC treatment *Hpa II*; 11: 50% FC treatment *Msp I*.

Data availability

The datasets used and/or analysed during the current study are included in this published article.

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Author contributions
Conceptualization, A.T., K.H., F.D., S.D., M.İ.I., A.A., M.T. and H.A.; methodology, A.T., K.H. and M.T.; software, A.T. and K.H.; validation, A.T.; formal analysis, A.T; investigation, A.T., K.H. and M.T.; resources, A.T., K.H., and M.T; data curation, A.T., K.H., M.T. and H.A; writing—original draft preparation, A.T., F.D., and S.D.; writing—review and editing, A.T. and H.A.; visualization, A.T., K.H., M.T. and F.D.; supervision, K.H. and M.T.; project administration, A.T., K.H. and M.T.; All authors have read and agreed to the published version of the manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

Permission for land study
The authors declare that all land experiments and studies were carried out according to authorized rules.

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