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Generation of a Potato Radiation Mutation System to Analyse the Features of Radiation Mutant RM1

Liqin Li [1,*], Linjuan Jiang [1,2], Bi Ren [1], Yifei Lu [1], Chengcheng Lv [1], Chenrui Wang [1], Liming Lu [1] and Shimin Yang [1,*]

[1] College of Agronomy, Sichuan Agriculture University, Chengdu 611130, China; juan498@163.com (L.J.); renbi1232021@163.com (B.R.); sarklu@126.com (Y.L.); chengchenglyu@163.com (C.L.); 17740960702@163.com (C.W.)
[2] College of Life Sciences, Northwest A&F University, Yangling 712100, China
* Correspondence: liliqin@sicau.edu.cn (L.L.); yangshimin1@163.com (S.Y.)

Abstract: Potatoes are widely planted worldwide and are the third most important food crop. Mutation breeding involves the artificial use of various physical and chemical factors to induce plants to produce new genotypes. In this study, we established a potato radiation mutation system and analysed the features of a radiation mutant. The LD50 of the potato callus was 24.8 Gy after linear regression analysis. The radiation mutant 1 (RM1) showed significant dwarfism and strong growth; RM1 plants decreased in height by 31%. The root and leaf fresh weights of the RM1 increased 1.8-fold compared to the wild-type (WT, Chuanyu 10) cultivar. Leaf microstructure results showed that the thicknesses of the upper epidermis and lower epidermis of RM1 plants were greater than those of the WT cultivar. Transcriptome analysis of seeding revealed 1179 upregulated and 1641 downregulated differentially expressed genes (DEGs) in RM1 plants. Further analysis showed that the expression levels of WRKY and MYB transcription factors, CIPK and MAPK protein kinases, ABC transporters, hormones, and ROS pathway genes were altered. These results provide theoretical support for potato breeding research and enrich the functional network of vital breeding-related genes.

Keywords: potato; 60Co γ-ray mutagenesis; seeding; transcriptome

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1. Introduction

As the third most important food crop, potato has been widely planted worldwide because of its short growth cycle, high nutritional value, and strong adaptability. To meet the needs of the potato industry, we urgently need to cultivate new varieties with disease resistance, stress resistance, high tuber yield and quality, and specific uses [1]. Compared with breeding of other annual crops, potato breeding work takes a long time because of the complexity of its genetic structure and its tetrasomic inheritance [2], and also the limitation of seed stock in the early stages, which also delays the breeding process. Cultivated potato is a tetraploid crop with a highly heterozygous genome. Inbreeding depression is a serious phenomenon. Because potato is an autotetraploid asexual breeding crop, traditional breeding methods have many limitations. The vast majority of potato varieties bred worldwide depend on traditional breeding techniques, and approximately 10 years is usually required to breed a new potato variety [3]. Mutation breeding involves the artificial use of a variety of physical and chemical factors to induce plants to produce new genotypes. These genotypes are screened according to breeding objectives to obtain new varieties. The offspring characteristics obtained by mutation breeding are relatively stable [4]. For example, a large amount of genetic variation materials (abnormal leaves, stems, flowers, and tubers) in diploid potatoes were obtained by using ethylmethanesulfonate mutagenesis [5]. In two cultivars (Atlantic and Desiree), silencing gene functions, such as vacuolar invertase and asparagine synthetase 1, at the same time were found to decrease the content of reducing sugars and asparagine by CRISPR-Cas9 technology [6].

Through mutation breeding, varieties that are not present in the natural world or are difficult to obtain by conventional breeding can be cultivated. Radiation mutation breeding has the advantages of increasing the gene mutation rate, breaking character linkages and promoting gene recombination, overcoming plant self-incompatibility, promoting gene transfer by distant hybridization, and shortening the breeding period [7,8]. Recent research has suggested that X-ray irradiation (>10 kGy) also improves the anti-retrogradation properties of starch in potatoes, especially at 30 kGy, and the anti-ageing property of X-ray-irradiated starch increases by 51%, which is conducive to maintaining the good taste and flavour of starch-based food during storage [9]. At present, 60Co γ-rays are the most widely used physical mutagen, and their breeding effect is remarkable. Radiation treatment can induce mutations in potatoes, and beneficial mutations can be fixed by asexual propagation, which can accelerate the process of variety selection. Reportedly, 60Co γ-ray mutagenesis has been effectively applied to improve potato characteristics such as disease resistance [10], heat resistance [11], salt tolerance [12], increased microtuber yield [13], reduced tuber glycoalkaloid content [14], and inhibited tuber sprouting [15]. A recent study has shown that 5 or 10 Gy radiation of nodal segments from shoots can enhance the yield and quality of tubers, such as high dry matter, low reducing sugar, and high starch content [16]. Therefore, using 60Co γ radiation to improve potato varieties is an effective breeding method.

Transcriptome sequencing is particularly important for understanding the gene expression patterns associated with specific phenotypes in potato plants. Li et al. reported that BR, GA, and ethylene pathway genes, such as CML13, ATP-dependent (S)-NAD(P)H-hydrate dehydratase, and Snakin 2, play key roles in potato tuber sprouting according to transcriptome analysis [17]. The transcriptome of a late blight-resistant variety (SD20) revealed that WRKY, ERF, MAPK, and NBS-LRR family genes are involved in the disease resistance process [18]. Subsequent WGCNA of potato RNA-Seq data revealed 116 differentially expressed hub genes involved in photosynthesis, nitrogen accumulation and metabolism, and secondary metabolites, as well as genes related to NRTs, auxin induction and regulation, NADPH, and wall-associated kinase [19].

Therefore, our research objective is to obtain mutants with growth advantages through 60Co γ radiation and enrich potato breeding resources. In this study, we established a potato radiation mutation system and obtained a radiation mutant (RM1); this mutant exhibited significant dwarfism and strength compared to the WT cultivar. Transcriptome analysis revealed 1179 upregulated DEGs and 1641 downregulated DEGs in RM1 plants after growing for 20 days on MS medium. Further analysis revealed that the expression levels of transcription factors, protein kinases, transporters, hormones, and ROS pathway genes were altered. Our results provide abundant gene resources for future breeding and lay the foundation for further revealing the functions of important genes.

2. Materials and Methods

2.1. Tissue Culture Method of Potato Stem Cells

A total of 0.5 cm of stem segments (without lateral buds) from potato seedlings (WT, cultivars Chuanyu 10) grown in test tubes for 20 days were cut and inoculated in MS callus induction medium (6 BA 1.5 mg·L^{-1} + NAA 0.4 mg·L^{-1} + 2,4-D 0.2 mg·L^{-1}; pH, 5.8; culture temperature, 22 ± 2 °C; dark culture for 7 days; light culture conditions, 1500–2000 μmol m^{-2}·s^{-1}, corresponding to strong sunlight light intensity; photoperiod, 14 h light/10 h dark). After 14 days of inoculation in a callus differentiation induction medium, the optimal combination of plant growth regulators in the callus differentiation culture was determined based on the Box–Behnken experimental design principle [20], with the concentrations of three plant growth regulators, TDZ, 2,4-D, and GA 3, as three factors. Hormonal reagents used were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Design Expert V8.0.6.1 statistical software was used for the experimental design and regression analysis.

2.2. Radiation Mutation of Seedings

In this study, the experimental design is as follows: using 60Co γ-rays as a radiation source, with an irradiation dose rate of 1 Gy·min^{-1}, four irradiation doses were applied, namely 0 (CK), 10, 20, and 30Gy. Each treatment group had 100 irradiated callus tissues, and treatments were repeated three times, so 300 calli in total were used. 60Co γ radiation treatment was carried out at the Radiation Center of the Institute of Biotechnology and Nuclear Technology, Sichuan Academy of Agricultural Sciences. Using SPSS software (v14.0), a linear regression was obtained between the survival rate of callus tissue (y) and radiation dose (x), and the regression equation was $y = -0.022x + 1.0467$. According to the regression equation, the LD50 is calculated to be 24.8 Gy. When adventitious buds on the irradiated callus tissue grew to 1–2 cm, they were removed and placed in a rooting medium (MS + 0.4 mg·L^{-1} NAA) for cultivation.

2.3. Preparation of Leaf Paraffin Sections

Leaves from 20-day-old WT and RM1 plants were selected, stored in FAA fixation solution, and sliced using the conventional paraffin sectioning method. The slices were observed under a NIKON ECLIPSE CI optical microscope and photographed using the NIKON DS-U3 imaging system. ImageJ software (v1.8.0), was used to measure leaf thickness, upper epidermal thickness, lower epidermal thickness, mesophyll palisade tissue thickness, spongy tissue thickness, and other indicators. Fifteen values were observed for each indicator, and then the average value was calculated.

2.4. Transcriptome Analysis

Seedlings of the WT and RM1 plants with a growth period of 20 days were frozen in liquid nitrogen, followed by storage at −80 °C. Total RNA was isolated using TRIzol (Invitrogen, Carlsbad, CA, USA). First-strand cDNA (complementary DNA) was synthesized using a QiaQuick PCR extraction kit (QIAGEN, Duesseldorf, Germany). Complete libraries were subjected to sequencing analysis. Clean tags were mapped to the potato reference genome and genes available at http://potato.plantbiology.msu.edu/pgsc_download.shtml (accessed on 9 January 2024) using the alignment software SOAPaligner/soap2 (BGI) (v2.0). DEGs were defined as genes with an absolute log2 ratio ≥ 1. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were used to determine the functional categories and metabolic pathways of the DEGs.

2.5. Quantitative RT–PCR Analysis

RNA was extracted from potato seedlings after they had grown for 20 days, and then cDNA was obtained using a RevertAid First-Strand cDNA Synthesis Kit (Invitrogen, Carlsbad, CA, USA). qRT–PCR was conducted using a Bio-Rad MiniOption System (Bio-Rad Laboratories, Hercules, CA, USA). The $2^{-\Delta\Delta Ct}$ method was used to calculate gene expression [21]. Elongation factor 1 alpha-like (EF1αL) was selected as a reference. The primer sequences are listed in Table S1.

2.6. Statistical Analysis

A one-way analysis of variance was used to test the effect of radiation treatment on callus tissue growth and to test the difference between radiation treatment and control for the survival rate of callus tissue. All data in this study were analysed using unpaired Student's t-tests with significance levels of $p \leq 0.01$ and $p \leq 0.05$. The data are shown as the means ± SEs ($n = 3$), and n represents the number of biological replicates. SPSS14.0 and Excel 2010 software were used for the statistical analyses.

3. Results

3.1. Establishment of the Stem Tissue Culture System

We selected virus-free, test tube-generated potato plants that had grown vigorously for 20 days, harvested stem segments measuring approximately 0.5 cm in length without lateral

buds, and inoculated them into a callus induction medium. After 14 days of cultivation, the plants were transferred to a bud induction medium. After 30 days of observation, the germination rates of 17 cultivars fluctuated between 51.98% and 91.21% (Table 1). According to the Box–Behnken experimental design principle, the optimal ratio of plant growth regulators in callus differentiation culture was determined: MS + 2.02 mg L^{-1} + TDZ + 0.08 mg L^{-1} 2,4-D + 2.25 mg L^{-1} GA 3 (Table 1). Stem tissues were cultured based on the above concentrations. First, stem segments from the test tubes were inoculated with callus tissue for culture for 14 days. The surface texture of the callus tissue was tight, and the structure was loose. After 30 days, obvious clustered buds were visible to the naked eye. After 40 days, the number of buds increased, and the buds attached to MS grew well after allowing 14 days for regeneration, showing a robust root system (Supplementary Figure S1). These findings suggested that the stem tissue culture system was established successfully.

Table 1. Box–Behnken design and results.

TDZ (mg·L^{-1})	2,4-D (mg·L^{-1})	GA_3 (mg·L^{-1})	30 D Shoot Regeneration Ratio/%	20 D Callus		30 D Cluster Buds	
				Colour	Quality	Stem	Leaf
2.50	0.10	5.00	87.01	green	loose	strong	fleshy
4.50	0.15	5.00	73.99	green	compact	weak	slender
2.50	0.05	0.50	91.21	green	loose	strong	curly
0.50	0.05	5.00	55.16	yellow green	little compact	strong	fleshy
0.50	0.10	0.50	51.98	white green	compact	strong	fleshy
2.50	0.15	9.50	79.93	light green	compact	weak	slender
4.50	0.05	5.00	81.06	green	little loose	weak	slender
2.50	0.05	9.50	77.94	yellow green	loose	weak	slender
4.50	0.10	0.50	88.16	green	loose	strong	curly
4.50	0.10	9.50	67.99	light green	compact	weak	slender
0.50	0.15	5.00	56.15	white green	compact	strong	fleshy
0.50	0.10	9.50	64.92	light green	compact	weak	slender
2.50	0.15	0.50	79.90	light green	compact	strong	curly

3.2. Radiation and Regeneration of Potato Stem Calli

The rate of normal seedling induction in the stem segments of the potato cultivar "chuanyu 10" cultured in an induction medium was greater than 95%. First, different irradiation doses (0, 10, 20, 30 Gy) of 60Co γ-rays were used to treat the stem segments. After 60 days, the growth of calli was obviously affected by different irradiation doses, and regenerated plants exhibited different growth states (Figure 1). With increasing radiation doses, the survival rate and germination rate decreased significantly, and the number of mutant plants decreased. The survival rate was 93%, and the budding rate was 85% when the plants were treated with 10 Gy (Figure 1B), and these rates were 64% and 54% when the plants were treated with 20 Gy and 30 Gy (Figure 1C,D). The difference in callus growth was obvious among the treatments with radiation doses of 10–20 Gy. Next, based on the correlation between callus survival rate (y) and radiation dose (x), the LD50 of potato calli was 24.8 Gy after regression analysis.

3.3. Detection of Morphological and Physiological Characteristics in RM1 Plants

The mutant produced after radiation was named radiation mutant 1 (RM1). After growing for 20 days, the plants in the RM1 treatment group had dark leaves and short heights (Figure 2A). The RM1 plants were 9.2 cm long, and the WT plants were 13.5 cm long; the RM1 plants decreased in height by 31%. The root and leaf fresh weights of the RM1 and WT plants were 2.95 g and 1.63 g, respectively, a 1.8-fold increase. Compared with that in the leaves of the WT plants (Figure 2B,C), the chlorophyll content in the leaves of the RM1 test tube plants increased significantly by 2.28-fold (Figure 2D). The leaf microstructure results revealed significant differences between the WT and RM 1 plants (Figure 3) in terms of the thicknesses of the upper epidermis, lower epidermis, palisade tissue, spongy tissue,

and leaves. The ratio of palisade tissue to spongy tissue, the tightness of leaf palisade tissue, and the looseness of leaf spongy tissue were significantly different (Table 2). The thicknesses of the upper epidermis and lower epidermis of RM1 plants were greater than those of the WT cultivar; the RM1 cultivar's thickness was 176.23 μm, while the WT cultivar's thickness was 123.84 μm, an increase of 42.30%. The thickness of RM1 plants' palisade tissue was 58.28 μm, and that of the WT tissue was 37.75 μm, an increase of 54.38%. The grid-to-sea ratio of RM1 plants was 0.73, while that of the WT cultivar was 0.63, an increase of 15.87%.

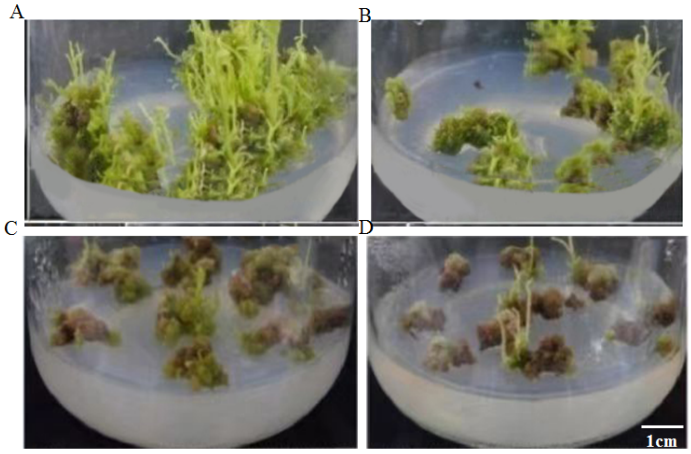

Figure 1. Effects of different radiation doses on potato callus tissue. (**A**) control; (**B**) plants with10 G treatment; (**C**) plants with 20 Gy treatment; (**D**) plants with 30 Gy treatment.

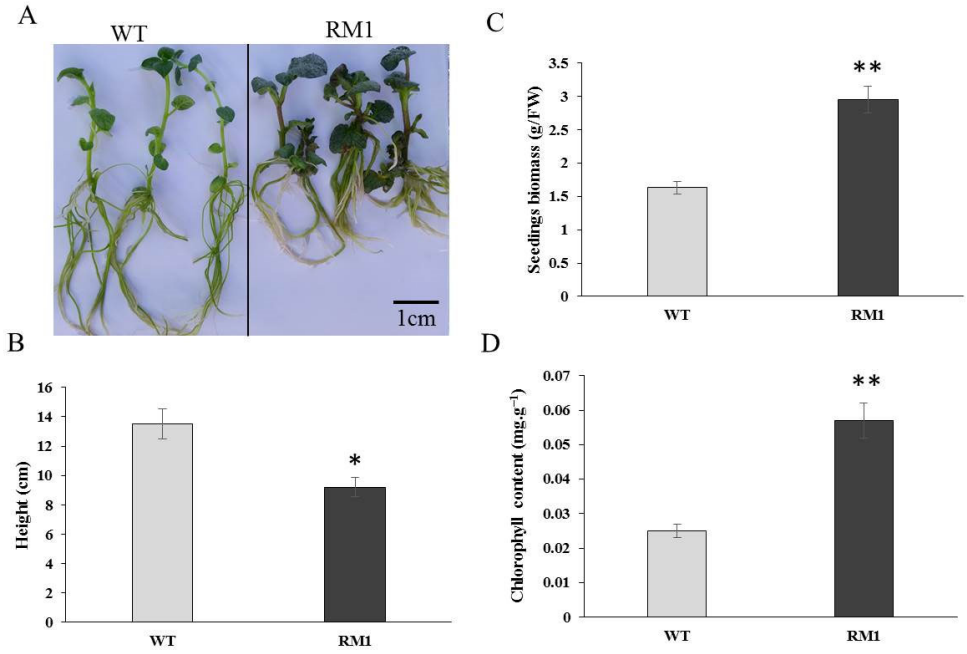

Figure 2. Growth status analysis of RM1 plants. (**A**) Seeding of WT and RM1; (**B**) measurement of height; (**C**) measurement of biomass; (**D**) measurement of chlorophyll content. Data are presented as mean ± SE of three biological replicates. Asterisks represent significant differences between control and gamma-irradiated plants (unpaired *t*-test: * $p < 0.05$; ** $p < 0.01$).

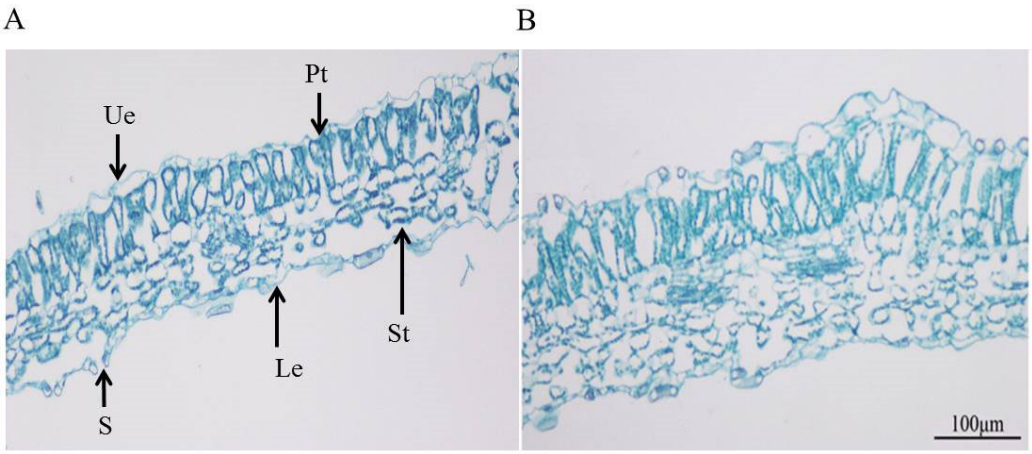

Figure 3. Observation of the leaf microstructure. (**A**) Leaf microstructure of WT; (**B**) leaf microstructure of RM1. Note: Ue: upper epidermis; Pt: palisade tissue; St: spongy tissue; S: stoma; Le: lower epidermis.

Table 2. Changes in microstructure of the leaf tissue of RM1.

Name	Ue Thickness (μm)	Le Thickness (μm)	Pt Thickness (μm)	St Thickness (μm)	Leaf Thickness (μm)	Pt/St	CTR	SR
WT	12.95 ± 1.40 b	12.08 ± 0.89 bc	37.75 ± 2.48 c	61.06 ± 2.42 f	123.84 ± 3.51 e	0.63 ± 0.04 b	0.30 ± 0.02 bc	0.49 ± 0.01 bc
RMI	20.16 ± 1.55 a	15.65 ± 1.05 a	58.28 ± 2.06 b	82.13 ± 2.89 e	176.23 ± 3.37 d	0.73 ± 0.04 a	0.03 ± 0.01 ab	0.47 ± 0.01 c

Note: Ue: upper epidermis; Le: lower epidermis; Pt: palisade tissue; St: spongy tissue; CTR: tightness of leaf palisade tissue; SR: looseness of leaf spongy tissue. Different lowercase letters show significant differences ($p < 0.05$) among samples.

In terms of the thicknesses of the upper epidermis, lower epidermis, palisade tissue, spongy tissue, and leaves. The ratio of palisade tissue to spongy tissue, the tightness of leaf palisade tissue, and the looseness of leaf spongy tissue were significantly different (Table 2). The thicknesses of the upper epidermis and lower epidermis of RM1 plants were greater than those of the WT cultivar; the RM1 cultivar's thickness was 176.23 μm, while the WT cultivar's thickness was 123.84 μm, an increase of 42.30%. The thickness of RM1 plants' palisade tissue was 58.28 μm, and that of the WT tissue was 37.75 μm, an increase of 54.38%. The grid-to-sea ratio of RM1 plants was 0.73, while that of the WT cultivar was 0.63, an increase of 15.87%.

3.4. GO and KEGG Analyses of Differentially Expressed Genes in RM1 Leaves

Two seedings' cDNA libraries (CK and RM1) were generated and sequenced using an Illumina HiSeq 2000 sequencer. The analysis revealed 1179 upregulated DEGs and 1641 downregulated DEGs in the RM1 plants compared to those in the CK plants (Table S1). After GO analysis, DEGs were classified into three groups—cellular component (CC), biological process (BP), and molecular function (MF)—and 33 functional groups at the second level (Figure 4). KEGG analysis suggested that the most represented DEGs were associated with metabolic pathways (497), biosynthesis of secondary metabolites (330), plant–pathogen interactions (153), plant hormone signal transduction, and phenylpropanoid biosynthesis (Figure 5).

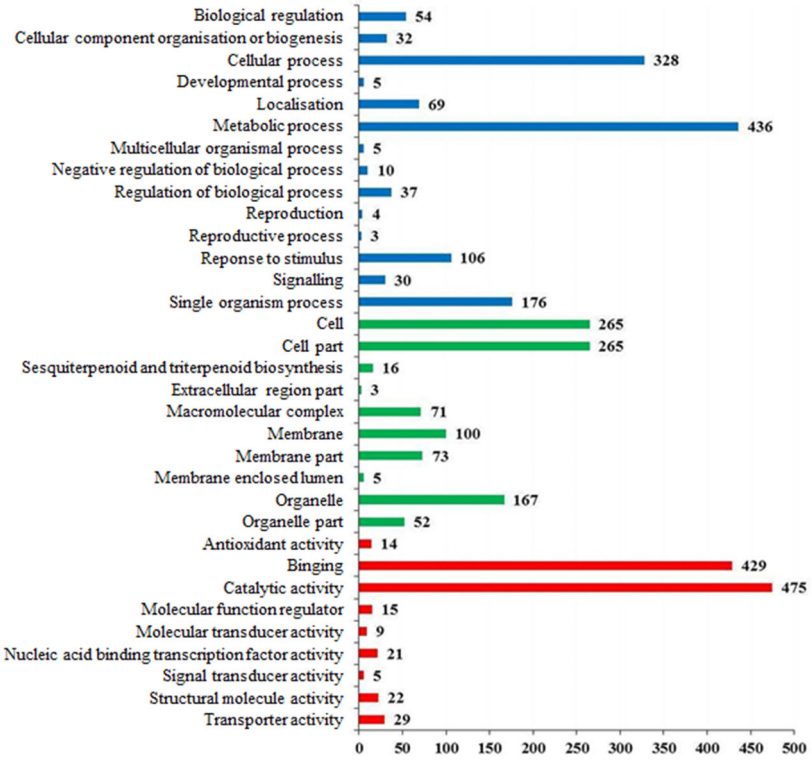

Figure 4. GO pathway analysis of the transcriptome. Note—blue: biological process; green: cellular component; red: molecular function.

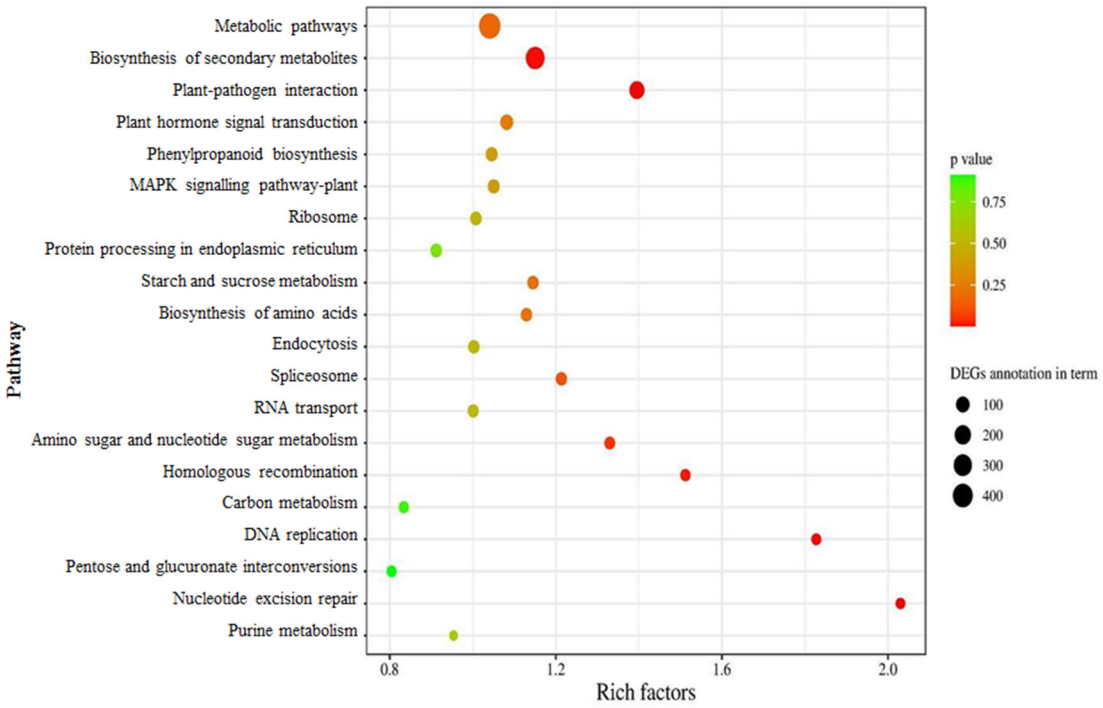

Figure 5. KEGG pathway analysis of the transcriptome. Note—red: upregulation; green: downregulation.

3.5. Verification of the Transcript Expression Levels of DEPs

A total of eight DEGs were selected to corroborate the accuracy of the transcriptome data using quantitative real-time PCR (qRT–PCR). Six gene expression patterns showed the same tendencies as those identified from the transcriptome data; for example, *the late blight-resistance protein R1A-3*, *abscisic acid receptor PYL9*, *wall-associated receptor kinase 5*,

and *catalase isozyme 3 (CAT3)* were upregulated according to the transcriptome data and qRT–PCR analysis in RM1 compare to WT, while *glutamate decarboxylase 4 (GAD4)*, *WRKY transcription factor 6 (WRKY6)*, and *GASA6* were all downregulated in RM1 compare to WT according to the two-method analysis. Only *ABCC11* showed opposite expression patterns according to the methods used. For example, the expression of *ABCC11* decreased 1.40-fold according to the transcriptome data but increased 1.54-fold according to qRT–PCR analysis. (Figure 6). These results suggested that the transcriptome data were reliable.

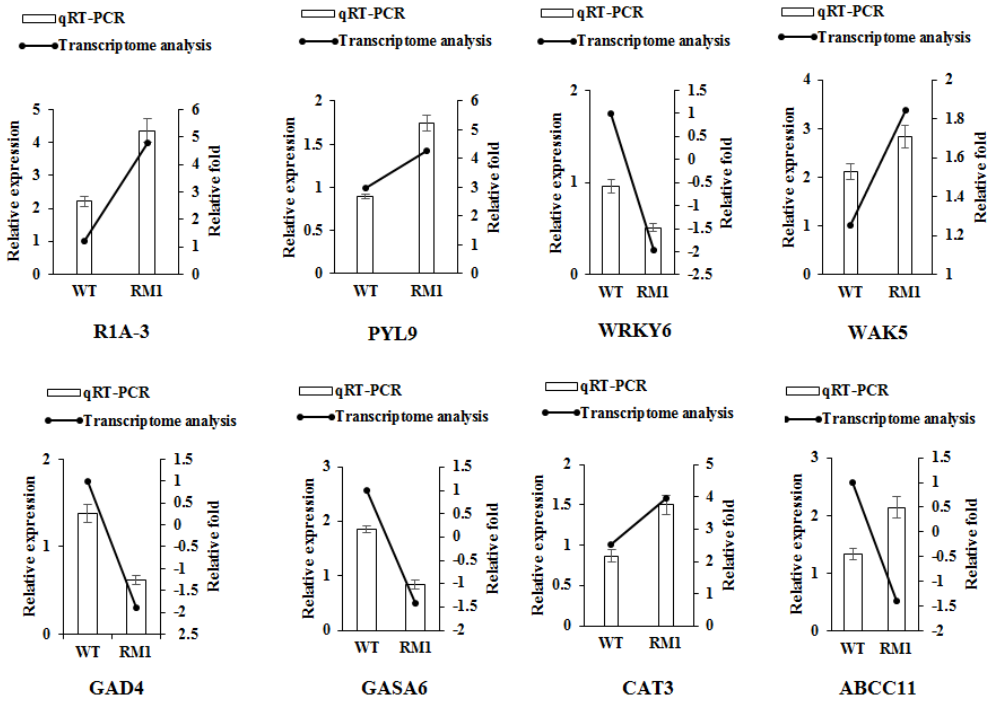

Figure 6. qRT–PCR analysis of the transcriptome. Note: R1A-3, late blight-resistance protein; PYL9, abscisic acid receptor; WRKY6, WRKY transcription factor 6; WAK5, wall-associated receptor kinase 5; GAD4, glutamate decarboxylase 4; GASA6, gibberellic acid-stimulated protein 6; CAT3, catalase isozyme 3.

4. Discussion

4.1. Mutagenic Effect of Radiation on Potato Plants

Plant calli are sensitive to radiation. Within a certain radiation dose range, irradiated plant bodies can emit signals to regulate certain processes, and gene variation frequently occurs during the process of repairing radiation damage [22]. By combining radiation breeding with tissue culture, new crop varieties with good characteristics can be selected. Safadi et al. reported that irradiation at 2.5 Gy—a low irradiation dose—led to a significant 38% increase in the number of microtubers [12]. Among tuber eye pieces subjected to 60Co γ irradiation at 35 Gy, two exhibited increased resistance to low-temperature sweetening and reduced levels of tuber glycoalkaloids [23]. Different potato varieties are susceptible to different radiation doses and have different breeding uses. In our study, the effect of radiation on potato calli was small at a dose of 10 Gy, but when the radiation dose exceeded 10 Gy, the survival rate and budding rate of potato calli decreased significantly with increasing radiation doses. 60Co γ irradiation was found to be an effective mutagen in RM1 plants subjected to treatment with 10 Gy, which is highly significant for plant phenotypic variation.

In this study, the thickness of the leaf epidermis and the grid-to-sea ratio increased significantly in RM1 plants, which had short stems (Figure 1). Large leaves were observed in potato mutants with high salinity tolerance [24] and in wheat mutants [25]. Wheat seed treatment with low-dose gamma radiation (10–100 kGy) reduced plant height and

improved plant vigour and flag leaf area, which may have contributed to improvements in plant photosynthetic productivity and dry matter accumulation in giant seeds [26]. Gamma irradiation applied to shoots at 20 Gy increased 20-hydroxyecdysone by 13-fold and induced metabolic changes in *Sesuvium portulacastrum* [27].

4.2. DEPs Related to Phytohormones

To better analyse changes in gene expression in RM1 plants, we conducted a transcriptome analysis of seedlings from WT and RM1 plants after they had grown for 20 days. The results revealed altered expression of 2820 genes, including many growth- and development-related genes, in the RM1 plants (Table S1), demonstrating that radiation mutagenesis successfully generated the RM1 line. Jong et al. reported that phytohormones are organic compounds produced in the plant body that can regulate (promote and inhibit) plant physiological processes. Auxin response factor (ARF) plays a central role in regulating auxin-induced gene expression. Transgenic tomatoes in which SlARF9 was silenced were larger than wild-type fruits; therefore, ARF9 negatively controls cell division during early fruit development in tomatoes [28]. Similar results were obtained in our research, which indicated that low StARF9 expression may have contributed to the strong growth observed in RM1 plants. The tryptophan aminotransferase (TSG1) gene encodes a key enzyme directly involved in auxin biosynthesis in rice that induced an increase in tiller number and a decrease in height [29]. Furthermore, TSG3 was decreased in our study, suggesting that it functions similarly to TSG1 to decrease GM1 height by affecting auxin biosynthesis. Zhao et al. reported that MsGH3.5 (indole-3-acetic acid (IAA)-amido synthetase)-overexpressing apple lines were dwarfed due to reduced free IAA contents [30]. StGH3.5 expression levels were also altered, possibly affecting the auxin and cytokinin pathways, which has substantial implications for GR1 seeding architecture. In potatoes, the deletion of gibberellin 3-beta-dioxygenase-2 (stga3ox2) affected plant height, and the resulting mutant was shorter than normal plants [31]. Overexpression of the gibberellin 2-beta-dioxygenase-1 (stga2ox1) gene in potatoes also resulted in a dwarf phenotype and reduced stolon growth during early development [32]. According to our data, the expression levels of GA2ox and GA3ox, which may contribute to reducing GA contents to cause dwarfing, were increased, which warrants further research in the future.

Nahirñak et al. reported that members of the gibberellic acid-stimulated Arabidopsis (GASA) peptide family were the most responsive to GA signals and that Snakin/GASA proteins play vital roles in plant growth, development, stress adaptation, and the disease resistance response [33]. We found that two genes, GASA6 and GASA10, were downregulated in RM1 plants. In Arabidopsis, GASA10 overexpression resulted in reduced silique elongation [34]. In rice, OsGSR1 (a GASA member), which is involved in interactions between the BR and GA pathways, could regulate the enzyme DIM/DWF1 to induce BR synthesis at the posttranslational level [35]. The serine/threonine protein kinase BRI1 is a vital component of the BR receptor complex, and *bri1* mutants have been reported to exhibit dwarfism and dark green leaves [36]. *BRI1* was downregulated in our study, and the RM1 phenotype was similar to the bri1 phenotype characterized by dwarfism and dark green leaves. We will perform related research in the next step.

4.3. DEPs Related to Signal Transduction Pathways

Plants have developed complex signal transduction mechanisms over the course of evolution, allowing them to sense external stimuli and transmit corresponding signals to elicit prompt responses to adversity and stress [37]. In plants, CBLs (calcineurin B-like proteins) are a unique type of Ca^{2+}-sensing protein that activate CIPKs (CBL-interacting proteins), which are Ser/Thr protein kinases, such as PKS (protein kinase S), with regulatory domains that decode Ca^{2+} signals. The interactions between CBLs and CIPKs form a complex signalling network with a major role in regulating various plant responses to stressors such as drought, low temperature, and saline–alkali environments [38]. When the expression level of CBL-interacting protein kinase18 (CIPK18) is upregulated, CIPK4,

CIPK7, and CIPK11 expression levels are downregulated. Mitogen-activated protein kinases (MAPKs) are essential for plant immunity and associated actions that protect plants from various environmental stressors [39]. MAP3K2, MAP3K3, and MAP3K13 were also increased in our study, and we hypothesized that these kinases could phosphorylate vital target proteins, thus altering the growth and development of RM1 plants.

The WRKY family consists of plant-specific transcription factors with important roles in various biological, metabolic, and response processes. WRKY6 positively influences senescence and pathogen defence by targeting PR1 and receptor-like protein kinases [40]. In Arabidopsis, WRKY6 regulates the expression of the PHO1 gene and participates in the plant response to low-phosphorus stress by binding to the W-box structure of the PHO1 promoter [41]. *WRKY6* and *pho1* expression levels were increased in RM1 plants, and high pho1 expression promoted phosphorus absorption to promote robust growth of RM1 plants. Furthermore, Ballachanda et al. reported that Arabidopsis plants in which RNAi was used to silence *WRKY75* expression were more susceptible to Pi stress and anthocyanin accumulation [42]. After growing for 20 days, RM1 seedling leaves were stored in the dark (Figure 3A), and transcriptome analysis revealed that *WRKY75* was downregulated, indicating that RM1 plants may have higher anthocyanin contents, although more experiments are needed for verification. *StMYBA1* overexpression in transgenic tobacco lines was reported to increase anthocyanin accumulation [43]. Additionally, the expression levels of two MYBs were increased, suggesting that WRKY75 and StMYBA1 function cooperatively to regulate anthocyanin accumulation in RM1 plants.

4.4. DEPs Related to Transporters

ABC transporters are mainly used by membrane integrins to transport substances, and substrate types differ according to the substance being transported, such as amino acids, sugars, lipids, alkaloids, organic acids, inorganic ions, and heavy metal chelates [44]. ABC transporter C family (ABCC) proteins participate in intracellular detoxification, chlorophyll metabolism, substance transport, and ion channel regulation [45]. AtABCC5 is located on the cytoplasmic membrane and serves as the central regulator of ion channels in defence mechanisms against abscisic acid and calcium signalling pathways in cells [46]. Burla et al. demonstrated that AtABCC1 and AtABCC2 in yeast can mediate ABA-GE transport into vacuoles and regulate the dynamic balance of ABA contents in cells [47]. In our study, ABCC14 and ABCC15 expression levels increased, and ABCC11 expression decreased. Further research is needed to determine whether these three proteins are involved in the calcium ion and ABA signalling pathways in potato plants. K^+ transporters and channels play vital roles in K^+ absorption and distribution in plants [48]. Potassium transporters (KTs) are involved in K^+ acquisition, redistribution, and homeostasis in plants [49]. Mhamdi et al. reported that the expression levels of *StNRT1.2*, *StNRT1.5*, and *StNRT2.1* were higher in N-efficient plant varieties than in other plant varieties, suggesting that NRT plays a positive and important role in nitrate accumulation and uptake [50]. In this study, StKT11 expression increased, and StKT6 expression decreased. Moreover, transcriptome analysis showed that the expression levels of the potassium channel AKT2/3 and some nitrate transporters were upregulated. Based on the above results, we hypothesize that these transporters and channels may increase K^+ and N absorption and utilization, which promote RM1 growth and developmental processes.

The signalling substance γ-aminobutyric acid (GABA) participates in various physiological processes related to plant growth and development. Under drought conditions, GABA synthesis and accumulation in leaves inhibited stomatal opening, thereby enhancing plant drought resistance [51]. GABA can be transported within and between cells [52]. Due to GABA transport from cytosol to mitochondria, the GABA transporter mutant AtGABP exhibits poor growth in carbon-deficient media and under low-light conditions [53]. In wheat, the expression levels of ALMTs (aluminium-activated malate transporters), which belong to a family that also includes anion channels, were negatively regulated by GABA in the presence of aluminium ions and under alkaline pH conditions [54]. In this study,

GABA transporter 2 was downregulated in RM1 plants, possibly increasing the extracellular GABA content; moreover, the extracellular GABA content in transgenic tobacco plants was found to be markedly higher than that in wild-type plants, which resulted in dwarfing of the stems of the tobacco plants [55], reflecting a phenotype similar to that of the RM1 plants. Here, we constructed a model to explain phenotypic changes in growth at the gene expression level (Figure 7). For example, WRKY and MYB transcription factors, CIPK and MAPK protein kinases, and ABC transporters play vital roles in RM1 growth and development.

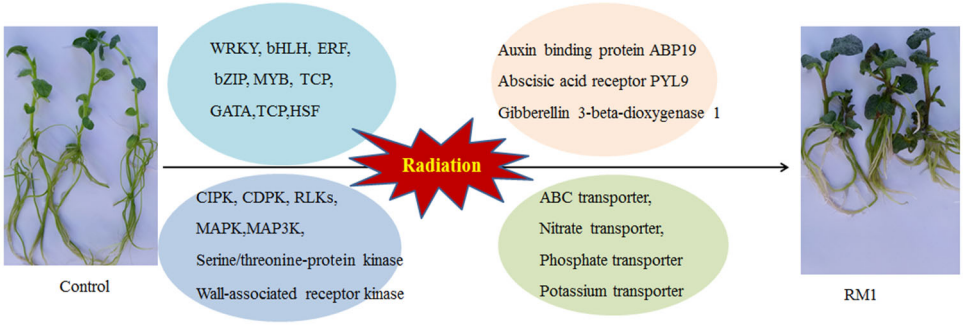

Figure 7. A working model of the transcriptional regulation of RM1 plants.

5. Conclusions

In our study, we first established a potato radiation mutation system of cultivars Chuanyu 10. After treatment with 10 Gy, the callus survival rate was 93%, and the budding rate was 85%. The LD50 of the potato callus was 24.8 Gy after analysis. This result provides a reference for radiation mutation dose for other potato varieties. The obtained radiation mutant RM1 showed a significant growth advantage (dwarf stems and dark leaves) compared to the WT cultivar. Leaf microstructure analysis revealed that the thicknesses of the upper epidermis and lower epidermis of RM1 plants were greater than those of WT plants. Transcriptome data revealed vital genes (WRKY and MYB transcription factors, CIPK and MAPK protein kinases, ABC transporters) playing necessary functions in RM1 growth and development. Subsequent research can determine their functions in growth and development through transgenic methods. This study also provides clues on exploring radiation breeding mechanisms and important genetic resources for potato breeding.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14071547/s1, Figure S1: Stem tissue culture of potato; Table S1: Information of differentially expressed genes; Table S2: Primers sequence of 8 gene were listed.

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