Article

"Exploring the Potential Role of Ribosomal Proteins to Enhanced Potato Resilience in the Face of Changing Climatic Conditions"

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Abstract: Ribosomal proteins (RPs) mediate protein synthesis and stability of the ribosomal complex. RPs and rRNA form ribosomal subunits and ribosomes, the cellular machinery for protein synthesis, a fundamental biological process related to cell growth and proliferation. The differences between paralogs within the same RP family have led them to acquire extraribosomal functions involved in plants' growth, development, and biotic and abiotic stresses. The transcriptomic analysis has unveiled essential role of the ribosomal proteins during microtuber development. The RPLs; RPL11, RPL29 and RPL40 interact with PEBP family members (Phosphatidylethanolamine-binding proteins), potential activators in the microtuber development. In potatoes, genome editing is a promising technology to introduce crop breeding traits. Recently, the edition of the genome through the CRISPR/Cas9 application has made it the unrestricted genetic modification method with the highest efficiency. Based on this, the gene modulation by overexpression and silencing in RPL11, RPL29 and RPL40 will guide us to understand the effect on the microtuberization process and produce improved potato plants with the capability of growing under adverse environments (biotic and abiotic stresses). By understanding the molecular biology mechanisms that RPs govern, we can improve crops under today's changing climatic conditions.

Keywords: Ribosome biogenesis; Potato; Carbon metabolism; Genome edition; CRISPR/Cas; Microtuberization; Potato

1. Introduction

Potato is the crop that produces the most significant amount of food per unit area, helping to fight global hunger and poverty. By 2050, the world population has been estimated to skyrocket to 10 billion. The agricultural production of food shall increase more than 60 percent to meet this rapidly growing demand. Climate change is a principal contributor affecting food production, while agricultural land expansion is not a sustainable solution to feed the world by 2050 [1]. Hence genetic modification of the potato, specifically of specific ribosomal proteins, represents one of the most promising alternatives for the present and the future.

The molecular mechanisms involved in potato tuber development under greenhouse conditions have been extensively studied [for review, see 2]. An alternative technology is the *in vitro* induction of potato microtubers (MTs). The axillary buds underwent MT induction in a medium containing

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high sucrose content, plant growth regulators, and several light quality/darkness. The benefits of MT production includes a higher multiplication rate, better storage and transport due to smaller size and weight, and easier, cheaper and faster cultivation than other methods [3 – 5].

A MTs induction protocol for potato *S. tuberosum* var Alpha by culturing stolon explants in MS medium supplemented with 8% sucrose, high content of gelrite 6.0 g/L, cytokinin (CK) 2iP 10 mg/L under darkness was developed by our research group. This protocol was based upon the underlying mechanisms of the interaction of CK signaling with homeobox transcription factors, RPs, cell cycle (CC), carbon metabolism, auxin-responsive factors and stem cell maintenance proteins also involved in the whole process [6].

Transcriptome analysis of the MTs revealed that ~1700 up-regulated and ~1600 down-regulated genes were regulating the morphogenetic process. The PPI network analyses were performed at highest confidence in the STRING database platform (v11.5, www.string-db.org), revealing ~300 genes were highly associated in tens of clusters. Two essential life proteins groups were discussed: Ribosomal proteins, of which RPL11 interacts with several PEBP family members, and proteins of the cell cycle [7].

The yeast two-hybrid approach for protein interactors screening found that the PEBP - StSP6A, a positive regulator of tuber development, can interact with RPs, other protein synthesis regulators, RNA and DNA interacting proteins, histones, initiation factors, flowering regulation, cellular signaling and carbon metabolism [8-12].

In the tuberization process of *S. tuberosum* from previous transcriptomic analyses have established the relevance of RPs during the tuberization process [13 - 17]. In the work of Sharma and Hannapel 2016 [13], a PPI network analysis revealed that RPs interact with SP6A, RP40SA, RP60S, RPS8, RPS4A, RPL10, BEL5, and RPL14. In addition, we applied the highest confidence value (0.900) in the same analysis, which showed 69 transcripts coding for RPs proteins that were up-regulated, 53 of which are directly involved in ribosome biogenesis [13].

In this manuscript, we will discuss the feasibility of activating RPs using CRISPR/Cas technology to induce MTs with several trait advantages such as size, number, stress tolerance, and protein content. We will also discuss the agronomic importance of several genes interacting with RPs derived from the transcriptome analysis performed by Valencia-Lozano et al. 2022 [7].

2. Transcriptome Analysis of MTs Induction

Transcriptome analysis of MT induction was performed according to Valencia-Lozano et al. 2022 [7]. The study revealed 1699 up-regulated genes, from which 299 were tightly associated with two essential biological processes and highly conserved through organisms: protein synthesis and cell cycle regulation comprising 29 and 117 proteins, respectively.

RPs interact with environment sensor proteins: 6 PEBP members, 21 related to osmotic stress, 9 with oxidative stress, 23 with CK response, 6 with one-carbon metabolism, 38 with carbon metabolism, 16 with TCA cycle, 6 with acyl carrier proteins, 14 fatty acid metabolism, 13 to thylakoid, 9 to redoxins, 8 with sulfur metabolism, 5 disulfide isomerase activity and 12 with immunophilins.

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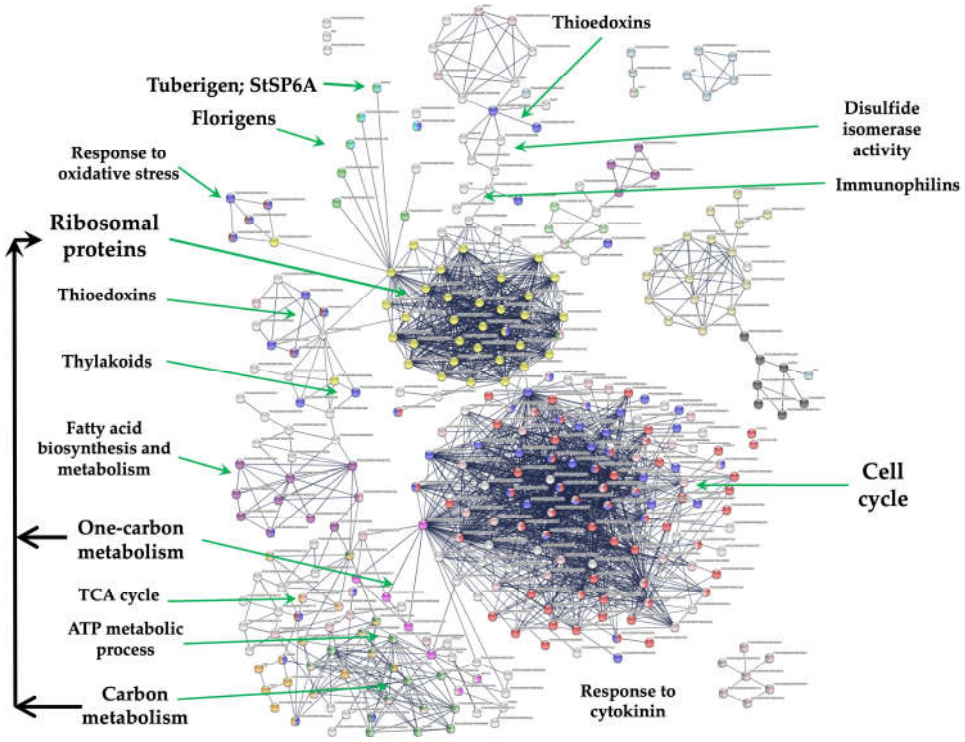

Figure 1. PPI network of RPL11 interacting with the tuberigen *StSP6A* and PEBP members during microtuberization of potato. Also, the interaction of RPL11 with thioredoxins, thylakoid biogenesis, fatty acid biosynthesis, and carbon metabolism. The PPI network has the highest confidence (0.900).

What is the Importance of Ribosome Biogenesis?

The biogenesis of the ribosome is directly associated with plant growth and development, and reproduction. The loss-of-function in genes encoding ribosomal proteins (RPs) or ribosome biogenesis factors (RBFs) gave rise to decreased growth, delayed flowering, and in more severe cases, are lethal. Loss-of-function of 19 ribosomal proteins and 26 ribosome biogenesis factors are seedling/embryo-lethal **(Table 1).**

Table 1. Loss-of-function of ribosomal proteins and chloroplast/ribosome biogenesis factors in plants.

RPS5A	Ribosomal protein. Arabidopsis	Growth retardation and floral and vascular defects and the recessive, embryo lethality	Weijers et al. 2001 [2]
RPS9	Ribosomal protein. Maize	Embryo lethal	Ma and Dooner, 2004 [3]
RPS13A	Ribosomal protein	Aberrant leaf and trichome morphology, retarded root growth and late flowering.	Ito et al. 2000 [4]
RPS16	Ribosomal protein. Arabidopsis	Embryo lethal	Tsugeki et al. 1996 [5]
RPS17	Ribosomal protein. Maize	Seedling lethal	Schultes et al. 2000 [6]
RPS18A	Ribosomal protein. Tobacco	Essential for survival, lethal.	Rogalski et al. 2006 [7]
RPS20	Ribosomal protein. Rice	Seedling lethal	Gong et al. 2013 [8]
RPS21	Ribosomal protein.	Decreased leaf pigmentation, plant growth and photosynthetic activity	Morita et al. 2004 [9]
RPS22	Ribosomal protein. Arabidopsis	No detectable alteration in growth	Tiller et al. 2012 [10]
RPS23	Ribosomal protein. Arabidopsis	Light-green phenotype and retarded growth severely disrupted mesophyll differentiation	Tiller et al. 2012 [10]

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RPS27	Ribosomal protein. Arabidopsis	Embryo lethal	Revenkova et al. 1999 [11]
RPL3	Ribosomal protein. Rice	Seedling lethal	Lee et al. 2019 [12]
RPL5C	Ribosomal. Arabidopsis	Embryo lethal	Dupouy et al. 2022 [13]
RPL9C, RPL9D	Ribosomal protein. Arabidopsis	Embryo lethal	Devis et al. 2015 [14]
RPL10	Ribosomal protein. Arabidopsis, Maize	Embryo lethal	Falcone et al. 2010 [15]
RPL11	Ribsomal protein. Arabidopsis	Significantly decreased leaf pigmentation, plant growth and photosynthetic activity	Pesaresi et al. 2001 [16]
RPL12	Ribosomal protein. Rice	Seeling lethal	Zhao et al. 2016 [17]
RPL13	Ribsomal protein. Rice	Embryo lethal	Lee et al. 2019 [18]
RPL15C	Ribosomal protein.	Embryo lethal	Bobik et al. 2019 [19]
RPL21C	Ribosomal protein. Arabidopsis, Rice	Embryo lethal	Yin et al. 2021 [20] , Lin et al. 2015 [21]
RPL23a	Ribosomal protein, ribosome biogenesis, Arabidopsis	RPL23aA RNAi: growth delay, irregularities in morphology of leaves, roots, phyllotaxy and vasculature, and loss of apical dominance	Degenhardt and Bonham-Smith, 2008 [22]
RPL24B	Ribosomal protein. Arabidopsis	Auxin-related developmental defects, in cotyledon number and vascularization	Zhou et al. 2010. [23]
RPL28-1	Ribosomal protein. Arabidopsis	Embryo lethal	Romani et al. 2012 [24]
RPL35-1	Ribosomal protein. Maize	Embryo lethal	Magnard et al. 2004 [25]
RPS20, RPL1, RPL4, RPL27 and *RPL35*	Ribosomal proteins. Arabidopsis	Embryo lethal	Romani et al. 2012 [24]
	Chloroplast/Ribosome biogenesis factors		
EDD1 (GlyRS9)	Glycyl tRNA synthetase. Arabidopsis	Embryo lethal	Uwer et al. 1998 [26]
CFG1, CFG2	Chloroplast development. Arabidopsis	Seedling lethal	Zhu et al. 2020 [27]
DCL-M	Defective *chloroplast and leaf-mutable. Tomato*	Embryo lethal	Bellaoui et al. 2003 [28]
CPN21	Chaperonin: Tomato, Tobacco	Seed abortion	Hanania et al. 2006 [29]
AtBRX-1-1, AtBRX-1-2	Maturation of the large pre-60S ribosomal subunit	Pointed leaf and delay early growth.	Weis et al. 2015 [30]
AtNuc-L1-AtNuc-L2	Ribosome biogenesis. Arabidopsis	Seedling lethal	Durut et al. 2015 [31]
AtTHAL	Nucleolar organization	*thal2* embryo lethal	Chen et al. 2016 [32]
AtNMD3	Nuclear export adaptor of 60S pre-ribosome export and maturation	Lethal	Chen et al. 2012 [33]
30 Ribosome biogenesis factors	Pre-rDNA transcription, pre-rRNA processing, modification, folding, and assembly with RPs	Gene disruptions: infertility, embryo lethality, impaired growth and gametophyte development, aberrant cotyledon, leaf and root development	Weis et al. 2015 [34]
RID1	DEAH-box RNA helicase, Pre-mRNA Splicing	*rid1-1*: abnormalities in shoot and root apical meristem maintenance, leaf and root morphogenesis	Ohtani et al. 2013 [35]
TIC32	Translocon of the inner envelope of chloroplasts	Embryo lethal	Hörmann et al. 2007 [36]
ATS2	Phosphatidic acid as intermediate for chloroplast membrane lipid biosynthesis.	Embryo lethal	Yu et al. 2004 [37]
TIC110	Translocon of the inner envelope of chloroplasts	Embryo lethal	Kovacheva et al. 2005 [38]

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CHL27	Chlorophyl biosynthesis	Retarded growth and chloroplast developmental defects	Bang et al. 2008 [39]
DG1	Early chloroplast development	Delayed greening phenotype	Chi et al. 2008 [40]
OEP80	Chloroplast Outer Envelope Protein	Embryo lethal	Patel et al. 2008 [41]
EMB5067/AKRP	Embryo development chloroplast protein	Embryo lethal	Garcion et al. 2006 [42]
SPC1	Carotenoid biosynthesis	Embryo lethal	Dong et al. 2007 [43]
PDS3	phytoene desaturase gene,	Embryo lethal	Qian et al. 2007 [44]
EMB1303-1	Chloroplast biogenesis	Embryo lethal	Huang et al. 2009 [45]
EMB1211	Chloroplast biogenesis	Seedling lethality	Liang et al. 2010 [46]
BPG2	Chloroplast protein accumulation induced by Brassinazole	Decreased number of stacked grana thylakoids	Komatsu et al. 2010 [47]
119 Nuclear genes-assoc. w/chloroplast	Embryo deffective mutants/ associated to chloroplast	Embryo lethal	Bryant et al. 2011 [48]
IRM	Involved in RNA processing	Embryo lethal	Palm et al. 2019 [49]
ZMRH3	*The RH3 DEAD Box Helicase*	Embryo lethal	Asakura et al. 2012 [50]
HSP90C	Chloroplast biogenesis	Embryo lethal	Inoue et al. 2013 [51]
FTSHI4	Thylakoid membrane-associated protein	Embryo lethal	Lu et al. 2014 [52]
RNAJ	Ribonuclease J (RNase J) required for chloroplast and embryo development	Embryo lethal	Chen et al. 2015 [53]
DER	Chloroplast ribosomal RNA processing	Embryo lethal	Jeon et al. 2014 [54]
Rrp5, Pwp2, Nob1, Enp1 and *Noc4*	Ribosome biogenesis factors	Embryo lethal	Missbach et al. 2013 [55]
SHREK1	Ribosome biogenesis factor	Embryo lethal	Liu et al. 2022 [56]
NOP2A,NOP2B	tRNA and rRNA methylation profiles	Embryo lethal	Burgess et al. 2015 [57]
RH22	RNA helicase22	Embryo lethal	Chi et al. 2012 [58]
MDN1	The AAA-ATPase MIDASIN 1 functions in ribosome biogenesis	Embryo lethal	Li et al. 2019 [59]

Lethal Mutants

The small subunits RPs, RPS1 and RPS20, are seedling-lethal in rice [18, 19], RPS9 and RPS17 are embryo lethal in maize [20, 21], RPS16 and RPS27 are embryo lethal in Arabidopsis [22, 23], and RPS18A is lethal in tobacco [24] (Table 1).

Large subunit RPs, RPL13, RPL12, RPL13, and RPL21C are seedling/embryo lethal in rice [25 – 28] (Table 1). In Arabidopsis, RPL5C, RPL9C,D, RPL10, RPL21C, and RPL28-1 are embryo lethal [29 - 34] (Table 1). At least 29 ribosome biogenesis factors have been shown to be lethal, including proteins involved in chloroplast development, ribosome biogenesis, nucleolar organization, and chlorophyll biosynthesis (Table 1).

In our transcriptome analysis 5 RPLs; RPL1, 12, 13, 27 and RPL35 and RPSs; RPS1, 9, 16 and RPS17 are embryo lethal.

Overexpression of Ribosomal Proteins and Ribosome Biogenesis Factors

Horvath et al. 2006 [35] demonstrated that *EBP1* transcriptional profile correlates positively with organ development and ribosome biogenesis related genes upregulation in potatoes. The EBP1 regulates the final stages of rRNA processing. Silenced potato lines showed reduced size, tuber yield and abnormal morphology.

Potato plants transformed with the RP StoL13a from *Solanum torvum*, SW, a highly resistant plant to *Verticillium dhaliae* infection, showed a resistance increase to *V. dahliae* infection compared with wild-type plants. The transformed plants exhibited reactive oxygen species decreased levels

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achieving an attenuated oxidative damage. In addition, several defence and antioxidant enzyme coding genes were upregulated in those transformed plants. These results support the plant defence role that StoL13a plays against *V. dahliae* infection [36].

Overexpression of RPL6 in Oryza sativa indica resulted in salt tolerance [37]. The overexpression of RPL23A in rice plants showed resistance to water deficiency, growth suitability and yield parameters compared to the negative control. It was also associated with upregulating many RPs, small and large subunits [38].

The 25S ribosomal RNA requires of the nucleolar methyltransferase activity of OLI2/NOP2A to process its transcript maturation and, in turn to join to the 60S large ribosomal subunit. Plants mutated in OLI2 yielded lighter seeds, displayed delayed germination, developed lesser lateral roots resembling the auxin-related mutants, and with increased sensitivity to sugar concentrations. The OLI2 overexpressing plants produced heavier seeds and showed a reduced sensitivity to high sugar concentration [39].

For the abiotic stress of cold the *STCH4/REIL2* triggers the upregulation of this ribosomal biogenesis factor. STCH4 overexpression in Arabidopsis gave rise to chilling and freezing tolerance. Also, the stch4 mutants showed a decrease in the C-repeat-binding factor (CBF) protein levels and, in turn, the expression of the genes induced by CBF was also decreased significantly [40].

Are RPs good candidate genes for improving of multiple abiotic stress tolerance in potato?

Crop plants, such as potato, are negatively affected by drought stress, for any developmental stage of the plant, from seed germination to tuber emergence and bulking to finally impact in tuber yield. Tuber cracking and malformation, hollow heart, vascular discolouration, and reduction in the dry matter accumulation in tubers are physiological disorders as a direct consequence of extensive periods of water deficiency.

Kappachery et al. 2013 [41] used the functional screening approach in yeast for the potential drought tolerance genes identification in potato plants. From potato plants exposed in a hyperosmotic culture media a cDNA expression library was constructed, and the transformed yeast with enhanced ability to survive under hyperosmotic stress expressing the cDNAs from the expression library were selected.

Sixty-nine genes were selected for their ability to grow under drought, salt, and heat stress. Of this, 8 were RPs; RPS7, RPL12, RPL10-like, RPL27, RPL18a, RPL1, RPS11, and RPL36 [41]. Accordingly, with that, the potential of RPs is very promising to obtain potato microtubers lines with desirable traits.

How to produce potato microtubers tolerant to biotic and abiotic stress?

The methodology CRISPR/Cas is a genetic modification tool that can be used to induce the expression of any gene, including the RPs. We found in the transcriptome analysis (RPL11, RPL29, RPL40, RPL16), interacting with several gene clusters, like the tuberigen and flowering, cell cycle, proteasome, immunophilins and oxidative stress.

The system CRISPRa 2.0 [42] and CRISPR-P 2.0 [43] can be used to obtain the plasmid constructs. Three guides RNAs (gRNAs) for the -228 bp and -19 bp from the transcription initiation site for each gene (for the avoidance TATA-box, initiator elements, and DPE), including those with the NGG sequence as PAM, aligning with the promoter region of the *S. tuberosum* genes *SP6A* (PGSC0003DMT400060057), *RPL11* (PGSC0003DMT400031869), *RPL29* (PGSC0003DMT400069470), *RPL40* (PGSC0003DMT400047686), *RPL16 (PGSC0003DMT400060127),* and cloned under methodology described by [44 – 45] (Lowder et al., 2015; Garcia-Murillo et al., 2023).

The vectors dCas9 fused to the VPG4, and the gRNA cassette, into a binary vector to generate: dCas9-VPG4-SP6A, dCas9-VP64-RPL11, dCas9-VP64-RPL2, dCas9-VP64-RPL40, dCas9-VP64-RPL16; named here SP6Av, RPL11v, RPL29v, RPL40v, RPL16v). Controls for transformation will be the empty entry vector containing only the dCas9 (dC9-v) and plant wilt-type without genetic modifications.

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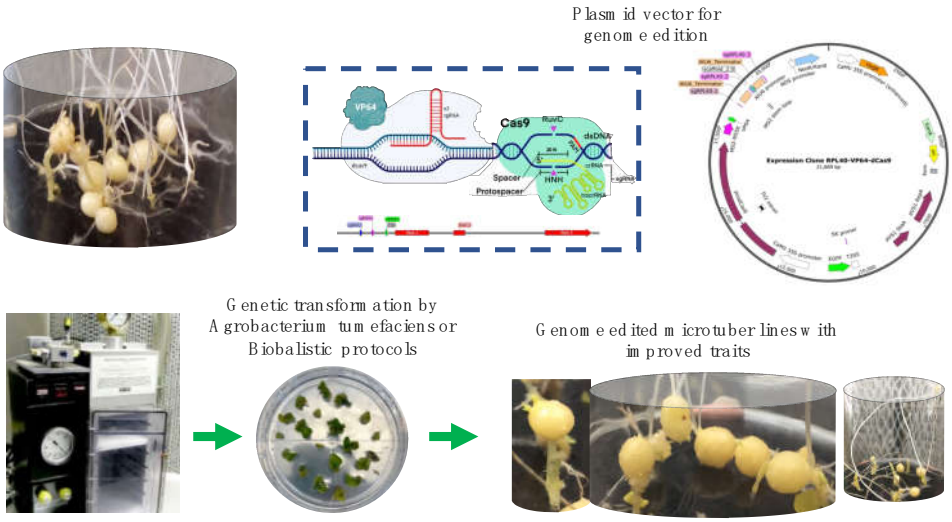

Figure 2. Genome edition strategy to obtain potato microtubers with improved traits.

The MTs test will be according to Herrera-Isidron et al., 2022 [6], and the protocol of genetic transformation and genome editing will be according to Cabrera-Ponce, et al. 1997 [46].

Interaction of RPs cluster with immunophilines

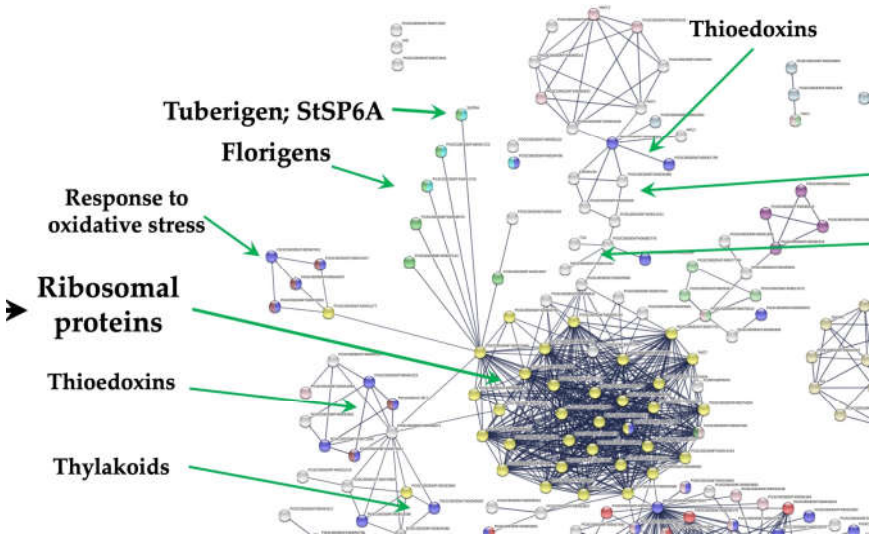

Figure 3. PPI network of RPs interacting with immunophilines, disulphide isomerase, thioredoxins, oxidative stress proteins during microtuberization of potato. The PPI network has the highest confidence (0.900).

RPL29 (PGSC0003DMT400069847) interacts with FKBP12. This group of proteins includes PPIs or immunophilins and protein disulfide isomerases. The cluster consists of 4 PPI genes interacting with SOS3 (PGSC0003DMT400023568), a calcium sensor calcineurin B, crucial for the salt stress-induced Ca2+ signalling transduction and activation of salt tolerance mechanisms in Arabidopsis. The SOS3 loss-of-function gave rise to salt hypersensitivity and significantly decreases the Ca^{2+} binding capability [47].

The *FKBP12* overexpression in Arabidopsis, has promoted growth under non stress conditions, and has increased the responses to abiotic stress [48]. Also, the CYP21 overexpression in potatoes, which is involved in oxidative stress response, produced longer plants, heavier tubers, and microtuberization yielded more significant amounts in a shorter time and weight per MT [49].

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Furthermore, three genes with Disulfide Isomerase-like proteins which are present in this cluster, STPDI1, 2 and 3. They interact with the calnexin (PGSC0003DMT400036920). StPDI1 accumulation triggers the quaternary structure establishment to counteracts salt exposure, enhance the catalyzes of disulfide bonds and contributes to reactive oxygen species response. Thus, they may work in a similar way as HSP for chaperone activity during salt stress [50]. The *StPDI1* expression levels reduction in potato plants negatively impacts the abiotic stress tolerance. The malate amount, succinate and 2-oxoglutarate and reducing equivalent NADH content was decreased significantly in StPDI1-inhibited potato plants. In contrast, serine and threonine content were significantly increased compared to wild-type plants [51].

Redoxins

RPs interact with the thioredoxin cluster through the interaction of the PRXQ peroxiredoxin (PGSC0003DMT400035271) with RPL11 (PGSC0003DMT400031869) and RPL18 (PGSC0003DMT400011931). This cluster consists of 7 peroxiredoxin genes. PRXQ carried out an oxidative stress protecting function through peroxides detoxification. And also, this protein is involved in the photosystem II protection against hydrogen peroxide.

To decrease the Reactive Oxygen Species (ROS) negative effects, aerobic organisms have developed counteracting mechanisms, involving catalases, superoxide dismutases, ascorbate peroxidases, glutathione peroxidases and GSTs with antioxidant enzymatic activities as well as the antioxidants synthesis molecules such as ascorbic acid and glutathione (GSH).

The transformed potato plants with the 2-cysteine peroxiredoxin with stress-inducible SWPA2 promoter or under the constitutive CaMV 35S promoter regulation showed a significant increase of tolerance to abiotic stresses, also including high temperatures and MV-induced oxidative stress. The 2-cysteine peroxiredoxin plants under the SWPA2 stress-inducible promoter exhibited the best tolerance [52].

Response to oxidative stress

The cellular production of Reactive O_2 species (ROS) are not restricted to an specific physiological status. Plants are the exception and have established physiological mechanisms to counteract ROS accumulation through a tight synthesis regulation and a plethora of enzymes for their remotion. The superoxide dismutases (SODs) in the cell are undoubtedly the first line of defence to prevent ROS accumulation.

The superoxidase dismutase 1 gene is essential in potato response to low temperatures, since its downregulation, through interference RNA, gave rise to a decrease in low-temperature tolerance and its overexpression impacts positively the tolerance to this stress in potatoes [53]. Furthermore, the SOD from *Potentilla atrosanguinea* (PaSOD) when is overexpressed in potato (*S. tuberosum ssp. tuberosum* L. cv. Kufri Sutlej) conferred a significant enhancement of the net photosynthetic rates (PN), as well as the stomatal conductance (gs) increase respect to wild-type plants (Pal et al. 2013). Interestingly, potato plants transformed to overexpress SOD, ascorbate peroxidase, and choline oxidase underwent an increase in the lignification process, starch synthesis, and several abiotic stress tolerance enhancement [54-55].

RPs interacting with Carbon metabolism, one-carbon metabolism, TCA-cycle.

In the PPI network, 38 genes were involved in carbon metabolism, 7 in one-carbon metabolism, and 6 in the citrate cycle. This cluster interacts with the CC cluster through the interaction of the DHFR and RPs.

Carbon metabolism

Carbon metabolism (CM) transforms carbon into energy in different amounts through glycolysis, gluconeogenesis, the pentose phosphate pathway, carbon fixation pathways, and the TCA pathway.

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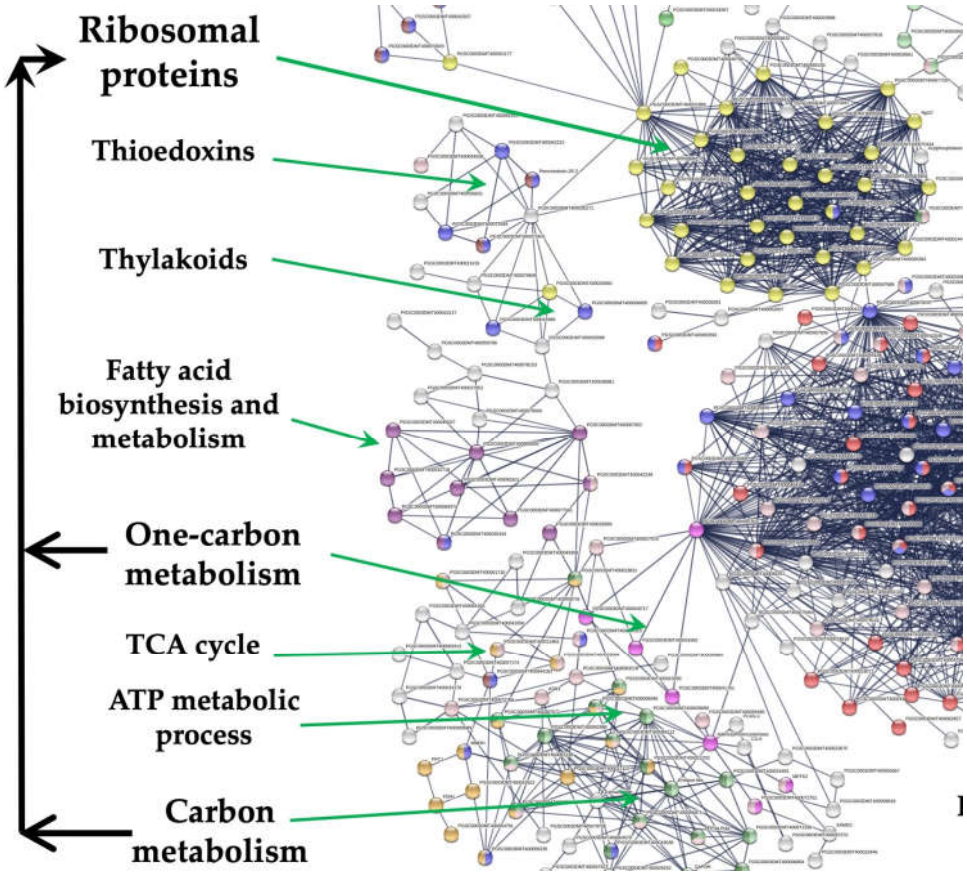

Figure 4. Cluster of genes directly related in carbon metabolism, one-carbon metabolism, TCA cycle interacting with the CC cluster and RPs.

Watkinson et al. 2006 [56] assessed three accessions-genotypes of *S. tuberosum ssp. Andigena* under drought stress conditions and through a transcriptomic analysis determined the relevance of carbon metabolism, citrate cycle and oxidative stress gene activation. In agreement with their results, we found similar genes compared with "intermediate" genotypes, with 20 genes upregulated under stress conditions, similar to our analysis. The term "intermediate" means plants that recovered the photosynthetic index after one cycle of stress and performed even better in the second, yielding similar results to the control plants unaffected by the stress. This may explain why molecular mechanisms involved in field conditions are very similar to those under *in vitro* conditions.

This includes PGK, Phosphoglycerate kinase, cytosolic; TPI, Triosephosphate isomerase, cytosolic; IAR4, Pyruvate dehydrogenase e1, GAPC2, Glyceraldehyde 3-phosphate dehydrogenase; LOS2, Enolase and PKP3, Pyruvate kinase. However, they showed that genes like Hexokinase-1 and Fructose-bisphosphate aldolase were down-regulated. In contrast, *IAR4* did not show significant changes in expression; otherwise, it did not happen when we induced stress. Regarding the TCA cycle, we found that the up-regulated genes, like Watkinson et al. 2006 [56], are MMDH1, Malate dehydrogenase and MDH, NAD-malate dehydrogenase. Overexpression of these genes have shown their agricultural relevance in different plant breeding programs. The phosphoglycerate kinase gene promotes biomass and yields in tobacco under salt stress conditions [57], while in rice, it improves thermotolerance [58]. Similarly, the overexpression of the pyruvate dehydrogenase in rice and barley plants impacted positively in drought stress tolerance, and grain size and weight [59-61]. Moreover, the overexpression of pyruvate kinase negatively affects root growth in maize [62], contrasting when it is silenced, in rice leads to sucrose translocation defects and grain filling inhibition [63] and decreases grain starch content [64]. Additionally, the triosephosphate isomerase gene overexpression in rice, pigeon peas, and maize plants enhance photosynthesis under elevated CO_2 levels [58], increases salt stress resilience, as well as better drought stress tolerance [65-67].

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Enolase contributes to salt stress tolerance, as determined in *Mesembryanthemum crystallinum L.* Likewise, the presence of the fructose-bisphosphate aldose gene is associated with salt stress response in *Brassica napus* [68] and tobacco [69], as well as biomass accumulation in tobacco [70]. The acetyl-CoA carboxylase overexpression leads to increased lipid content in microalgae, including *Dunaliella sp.* [71], *Chlamydomonas reinhardtii* [72], and *Scenedesmus sp.* [73], and increased seed yield in tobacco [74].

The sucrose phosphate synthase overexpression enhances growth, thermotolerance [75, 76], sink strength in tomatoes [77], potato yield characteristics [78], and cold tolerance in Arabidopsis [79]. It also positively impacts biomass production in sugarcane [80] and in tomatoes under saturating light and CO_2 conditions [81] and also enhances foliar sucrose/starch levels in Arabidopsis [82]. Similarly, the ATP-citrate synthase gene is directly involved in the salt stress response in *Halogeton glomeratus* [83] and sugar beet [84].

The glyceraldehyde 3-phosphate dehydrogenase overexpression enhances drought tolerance in potato [85] and salt tolerance in soybean [86], rice [87], and potato [88]. The 6-phosphogluconate dehydrogenase overexpression gene contributes to resistance against Nilaparvata lugens in rice [89], as well as salt tolerance in barley [90], and is also involved in starch accumulation in maize [91]. Additionally, malate dehydrogenase gene overexpression boosts organic acid synthesis and aluminium tolerance in alfalfa [92], salt tolerance in rice [93], apple, and tomato, along with cold tolerance [94, 95]. However, it is worth noting that the gene is embryo-lethal in Arabidopsis [96].

Lastly, the overexpression of the phosphoenolpyruvate carboxylase gene increases photosynthetic efficiency in rice [97], fatty acid production in *N. tabacum* [98], protein content in *Vicia narbonensis* [99], and affects dark and light respiration in potato [100].

In cerium stress-treated microalgae, *Nannocloropsis oculata* gave rise to lipid accumulation with the carbon metabolism prominently activated and ribosome biogenesis genes [101].

In summary, overexpressing specific genes for CM in different plant species improves stress tolerance, yield, biomass production, and metabolic processes.

One-carbon-metabolism

One-carbon metabolism is a metabolic process in which multiple enzymatic reactions provide methyl groups (one carbon) for nucleotide metabolism, purine and pyrimidine synthesis, and amino acid metabolism. These effects involve many cellular activities, such as cell growth, differentiation, and development. One-carbon metabolism carries out the mobilization of a carbon unit to the tetrahydrofolate (THF) from serine or glycine to yield methylene-THF for DNA synthesis.

The S-adenosylmethionine synthetase (*SHM4* and *SHM1*) overexpression enhances cold and salt tolerance in tobacco [102, 103], lipid production in Chlamydomonas [104], salt, H_2O_2 and drought tolerance in Arabidopsis [105, 106 Kim et al. 2015), and alkali tolerance in tomato [107]. The adenosylhomocysteinase gene (*HOG1*), when overexpressed, affects early flowering and reduced biomass in Arabidopsis [108], and it increases lycopene and reduces the ripening time in tomatoes [109]. Furthermore, the overexpression of the serine hydroxymethyltransferase gene (MAT3) confers salt tolerance [110], increases root growth and sugar levels and decreases H_2O_2 levels in Arabidopsis [111], cold tolerance [112] and antioxidant ability in rice [113].

Lastly, the DHFR-TS/THY-1, which is bifunctional dihydrofolate reductase-thymidylate synthase, can regulate folate abundance and also regulates ROS removal in Arabidopsis [114], essential for the biosynthesis of nucleotide precursors of DNA [115], and in somatic embryos in carrot [116].

3. Conclusions

1.- Gene modulation of ribosomal proteins by genome edition mediated by CRISPR/Cas technology can potentially enhance potato challenge to stress and increase nutritional value.

2.- The sets of genes involved in carbon metabolism: *PGK*, *TPI*, *IAR4*, *GAPC2*, *LOS2*, and *PKP3* that match with plant field experiments and our conditions have the potential to improve biomass and yield.

3.- The cluster of immunophilins and disulfide isomerase interacting with RPs will allow the activation of alternative mechanisms for survival enhancement under adverse conditions.

4.- The gene modulation of one-carbon metabolism pathways will favour survival under adverse environments.

4. Patents

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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