Brief information about the project

Name of the project	AP09259754 «Molecular characterization of the *Triticum aestivum* TORC1 signaling pathway and their significance in control of wheat seed germination». (0121PK00273)
Relevance	TOR signaling regulates protein translation and is one of the central hubs that integrates external and internal inputs to development and growth processes, therefore, the knowledge of the regulation of this essential and complex network in the early seedling growth is indispensable for breeding and biotechnological purposes. To date despite on the progress in total wheat genome sequencing (IWGSC RefSeq v1.0), the TOR protein itself and basic components of TORC1 signaling pathway in wheat still has yet to be detected. Seed germination is not only a critical developmental step in the life cycle of plants but is also important for agricultural production. Dry seeds are heterotrophic and contain all the components that are required for germination and seedling establishment until the seedling reaches the autotrophic state. The seed is filled with bioactive molecules such as proteins and mRNAs. These stored mRNAs are selectively translated during seed germination and post germinative seedling growth. At the early stage of germination, gibberellic acid (GA) stimulates *de novo* synthesis of proteases and peptidases and ∼50% of the total protein synthesis. Abscisic acid (ABA) represses most effects of GA, including GA-induced protein synthesis. During mRNA translation, the majority of actively translating ribosomes exist as polysomes in cells with multiple ribosomes loaded on a single transcript. Indeed, GA-dependent significant increases the number of ribosomes and polysome formation in the barley aleurone cells originally was detected in studies in the 1970s. Following this finding there were no active studies on the molecular mechanisms of GA-induced formation of polysomes in cereals. Recently, we have isolated cDNA gene of TaS6K1 for the first time, an uncharacterized component of TOR signaling pathway in wheat. Interestingly, phosphorylation of TaS6K1 on Ser-467 are strongly induced in the presence of a phytohormone - gibberellic acid. Besides, in our study the TOR inhibitors were selectively targeted the GA-dependent gene expression, including α-amylase, without affecting the ABA-dependent TaABI5 gene expression. These results strongly suggest that a growth of wheat seeds is dependent on TaTOR signaling. Based on these data we propose that GA-dependent activation of TaTOR-S6K1 play important role in transition from seed to seed germination. This proposed project is designed to further elucidate the role of TOR signal transduction pathway in GA-dependent regulation of wheat seed germination.
Purpose	In the present project we are planning isolation and molecular characterization of the wheat putative genes encoding components of TORC1 signaling pathway and their possible role in regulation of wheat seed germination. Knowledge of role this complex network in regulation of plant growth is essential for breeding and biotechnological purposes.

Objectives	1. Isolation and molecular characterization of the *Triticum aestivum* putative genes encoding components of TORC1 signalling pathway, i.e., TOR, Raptor, and LST8 and their expression analysis. 2. Characterization of the plant TOR signalling complex by defining its components and perform the functional studies of the pTOR complex. 3. Study of role TaTOR signaling in hormone dependent regulation of protein translation at the early stage of wheat seed germination.
Expected and achieved results	According to the results of the study, putative homologous genes encoding the TOR protein were identified by utilizing the recently completed wheat genome. The core components of TOR complex including TOR, RAPTOR, and LST8 are highly conserved in wheat. MALDI-TOF MS analysis and western blotting with the antibodies specific to wheat Target of Rapamycin (TaTOR) revealed presence of wheat target of rapamycin TaTOR in wheat embryos and aleurone layers. Yeast two-hybrid analysis and in vitro pull-down assay indicate that TaS6K1 and TaTOR physically interact with TaRaptor protein. Complementation tests of wheat LST8 protein in yeast showed that TaLST8 partially complemented a lst8 yeast mutant. Expression analysis showed that TaTOR, TaRaptor and TaLST8 were expressed in all tissues analyzed. It was shown that, rapamycin and torin 1 treatment resulted in an augmentation of the 80S peak and a corresponding reduction in the polysome peak. These alterations suggest a decline in mRNAs bound to polysomes and a simultaneous increase in mRNAs bound to monosomes or free mRNAs, indicating a widespread inhibition of translation. Furthermore, sequencing of polysome bound RNA revealed that rapamycin and torin 1 treatment significantly suppress expression of 220 genes, including those encoding cellular components protein of Photosystem I and II, plastid, vacuole, cell wall and proteins of xyloglucan metabolic, carbohydrate metabolic, photosynthesis, xyloglucosyl transferase activity processes and etc. These observations suggest that plant TOR plays an important part in the regulation of translational processes just as in other eukaryotes.
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List of publications with links to them	1. Smekenov I.T., Raike T.M., Tilvaldieva S.V. Polyclonal antibodies to the recombinant homologue LST8/GBL of *Triticum aestivum*, a component of the TORC1 signaling system [Rus: Poliklonal'nyye antitela k rekombinantnomu gomologu LST8/GBL *Triticum aestivum*, komponentu TORS1 signal'noy sistemy] // Experimental Biology – 2021. – Volume 88, No. 3. – P.96-107. 2. Bissenbaev A.K., Alybaev S.D., Smekenov I.T., Kolbaeva G.A. Participation of the TOR/S6K1 signaling system in the regulation of α-amylase gene expression and wheat grain germination [Rus: Uchastiye TOR/S6K1 signal'noy sistemy v regulyatsii ekspressii genov a-amilazy i prorastaniye zerna pshenitsy] // All-Russian scientific conference with international participation, and school of young scientists. Experimental plant biology and biotechnology: history and outlook into the future. Physiology and biotechnology of photosynthetic and heterotrophic cells. Moscow. - 2021. – P.304. 3. S. Alybayev, I. Smekenov, A. Kuanbay, D. Sarbassov, A. Bissenbaev. Gibberellic-acid-dependent expression of α-amylase in wheat aleurone cells is mediated by target of rapamycin (TOR) signaling // Current Plant Biology. – 2023. – Vol. 37, - Article number 100312. Doi: https://doi.org/10.1016/j.cpb.2023.100312 (Web of science: Q1; Scopus: percentile – 92%).
Patents	-