

WRKYs in Durum wheat: Intrinsic disorder and interactions

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Abstract

The WRKY transcription factors are involved in a range of biological processes in plants, including the response to biotic and abiotic stresses and plant immunity. There is also evidence that intrinsic disorder proteins (IDPs) are involved in key cellular functions such as regulating biological processes. Here, the intrinsic disorder distribution of WRKYs within *T. turgidum* and its protein-protein interactions are investigated. The analysis showed that the hub proteins have higher level of disorder content in *T. turgidum*. In comparison to other cereals, it is shown the intrinsic features in *T. turgidum* are evolutionary conserved, which may explain the multifunctionality of WRKYs in plants.

Keywords Durum wheat; intrinsic disorder; protein-protein interaction; WRKY.

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1 Introduction

Intrinsically disordered proteins (IDPs) have no defined tertiary structure, but they play key biological roles including regulatory signalling and transcription networks that involve hubs with multiple protein partners (Ward et al., 2004; Dunker et al., 2005). Plants use disordered proteins to establish quick and efficient mechanisms of adaptation to environmental constraints (Pazos et al., 2013). Large scale analysis of IDPs in cereals have shown that roughly 30% of their proteomes are mostly disordered and mainly involved in cellular and metabolic processes regulation and responses to stimulus (Choura et al., 2019-2021).

Transcription factors are modular proteins that contain at least one DNA-binding domain, recognize and bind to specific DNA sequences, involved in transcription regulation. Previous report indicated that the AT-hooks and basic regions of the DNA-binding domains of the transcription factors present a high level of disorder (Liu et al., 2006).

The WRKY proteins are transcription factors characterized by their highly conserved DNA-binding domain (DBD), known as the WRKY domain. WRKYs participate in biological processes such as the response to biotic and abiotic stresses (Ramamoorthy et al., 2008).

WRKY proteins with 2 WRKY domains belong to group I, while the majority of proteins with one WRKY domain are part of group II. Both group I and group II proteins share the similar pattern of potential zinc ligands (C-X4-5-C-X22-23-H-X1-H). Although WRKY proteins have only one WRKY domain, various zinc finger motifs are classified in Group III (Eulgem et al., 2000).

Recently, the distribution of WRKYs intrinsic disorder in *O. sativa*, *S. bicolor*, *Z. mays*, *T. aestivum*, *H. vulgare* and *A. thaliana* and their protein-protein interactions have been studied (Choura et al., 2020). This work focused on the intrinsic disorder of the WRKY transcription factors in durum wheat.

2 Material and Methods

2.1 Data sources

The dataset was retrieved from Uniprot database release 2022_01.

2.2 Protein disorder prediction

Disorder prediction and linear interacting peptides (LIP) were reported from the MobiDB database. MobiDB provides information about intrinsically disordered regions (IDRs) and related features from various sources and predictive tools (Piovesan et al., 2021).

2.3 Protein-protein interaction network

The protein-protein interactions network of WRKYs in *T. turgidum* was obtained by using *T. aestivum* as a background organism from STRING version 11.5 database (Szklarczyk et al., 2021)

3 Results and Discussion

The disorder content and linear interacting peptides (LIPs) of WRKYs in *T. turgidum* are listed in the Supplementary File. The data show that the mean content of disorder (25%) and of disordered binding regions between WRKYs analysed in this study are similar to those previously found in *O. sativa*, *S. bicolor*, *Z. mays*, *T. aestivum*, *H. vulgare* and *A. thaliana* (Choura et al., 2020). By grouping proteins according to the percentage of predicted disorder of their sequence, we found that the higher disorder content of WRKY proteins (87%) is within the interval 30-60%.

The protein- protein interaction network is shown in Fig. 1. The topological features of this network such as betweenness revealed that the highly connected nodes called hubs have a higher disorder and high linear interacting peptides (Table 1 and Supplementary File). This may explain the binding with many partners allowing thus multiple functions. These results are in good agreement with those found in *O. sativa*, *S. bicolor*, *Z. mays*, *T. aestivum*, *H. vulgare* and *A. thaliana* and consolidate the disorder conservation in WRKY family notably in cereals and *A. thaliana* (Choura et al., 2015, 2020).

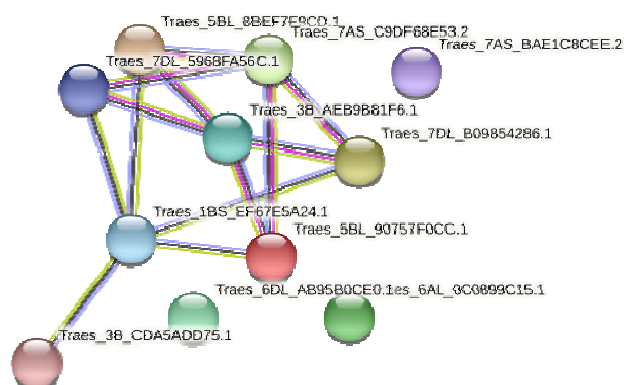
Moreover, the enrichment analysis of the network revealed that the GO functions underlying the WRKY proteins in durum wheat are particularly related to regulation of antifungal innate immune response, transcription regulation, responses to chitin, to salicylic acid and fungus.

4 Conclusions

This study has investigated the intrinsic disorder and protein- protein interaction network in the WRKYs in durum wheat. Compared to the others cereals, similar and conserved disorder features were found in durum wheat. The hub proteins having higher disorder content deserve experimental validation to uncover their multiple biological functions notably in stress responses.

Table 1 Network parameters of WRKY network in *T. turgidum*.

number of nodes	11
number of edges	13
average node degree	2.36
avg. local clustering coefficient	0.09

**Fig. 1** WRKY proteins network in *T. turgidum*.

Supplementary File

WRKYs in *T. turgidum*. It contains: 1- the UniProt identifiers, the disorder contents and the numbers of linear interacting peptides (LIPs), 2- mapping identifiers, and 3- Betweenness.

Entry	Status	Gene names	Length	disorder%	LIP%
A0A446N126	unreviewed	TRITD_3Av1C	1007	0	0
A0A446N180	unreviewed	TRITD_3Av1C	1011	0	0
A0A446N161	unreviewed	TRITD_3Av1C	1006	0	0
A0A446N153	unreviewed	TRITD_3Av1C	957	0	0
A0A446JKT2	unreviewed	TRITD_1Bv1C	334	30,2	23,1
A0A446PG53	unreviewed	TRITD_3Bv1C	490	19,6	33,7
A0A446NC20	unreviewed	TRITD_3Av1C	575	35,3	45,6
A0A446VPW	unreviewed	TRITD_6Av1C	553	37,4	51,4
A0A446WMN	unreviewed	TRITD_6Bv1C	560	33,8	49,5

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