**An analysis of the roles of reactive oxygen species and RBOHD in Arabidopsis immune response**

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**The immune response in plants differs from that of animals. Plants are capable of recognizing pattern recognition receptors (PRRs) that are activated via pattern triggered immunity (PTI); however, plants also rely on reactive oxygen species (ROSs) in order to be alerted to potential pathogens. Respiratory oxidase homologs (RBOHs), a form of NADPH oxidases, are the producers of ROSs during PTI. A relevant type of RBOH in Arabidopsis is RBOHD. *pbl13* Arabidopsis mutants show increased resistance to bacterial infection, but the mechanism remains unclear. The research indicates that PBL13 is negatively regulated via a RING domain E3 ubiquitin ligase (PIRE) and is, in part with PIRE, responsible for phosphorylation of RBOHD resulting in increased ROS production.**

**Introduction**

Plants, like animals, are capable of immune responses that enhance their resistance to certain pathogens. One of the main challenges for plants, however, is their inability to differentiate beneficial microorganisms from pathogens (Zhou and Zhang, 2020). A universal term among all eukaryotes is PTI, which stands for pattern triggered immunity (Albert et al., 2020).

PTI allows eukaryotic organisms to engage pattern recognition receptors (PRRs) in order to sense pathogen-associated molecular patterns (PAMPs) and microbe-associated molecular patterns (MAMPs) (Albert et al., 2020). When PRRs are activated, downstream signaling is triggered and reactive oxygen species (ROSs) are released (Lee et al., 2020).

ROSs are molecules that contain oxygen and possess a higher chemical affinity than O2 making them less stable; some of the most common ROSs in plants are O2, O2-, H2O2, and OH- (Waszczak et al., 2018). ROSs are mainly produced and contained in the chloroplasts, mitochondria, and peroxisomes (Das and Roychoudhury, 2014).

NADPH oxidases are responsible for ROS production during PTI (Lee et al., 2020). Lee et al., 2020 also states that a variation of NADH oxidases, called respiratory oxidase homologs (RBOHs), are responsible for pathogen defense among other functions in plants.

*Arabidopsis thaliana*, a commonly researched plant, has a genome made up of 10 genes that code for RBOHs (Waszczak et al., 2018). The most relevant isoform of RBOH is called RBOHD and is in charge of PAMP activation and ROS production (Lee et al., 2020). The previously cited study indicates that once PAMP is activated in Arabidopsis, conformational changes in the N-terminal of RBOHD occur due to the rapid entry of Ca2+ ions resulting in production of ROS. It is also believed that the relationship between calcium signaling and ROS production is mutually activating (Zhou and Zhang, 2020).

It is understood that *pbl13*, an Arabidopsis mutant, shows increased resistance to *Pseudomonas syringae* and greater ROS production via negative regulation; however, the mechanism of how this occurs is still unknown (Albert et al., 2020, Lee et al., 2020).

Negative regulation of immunity is crucial in plants because it certifies that the necessary response is carried out when a plant is exposed to a pathogen (v). The primary research cites that the three common types of negatively regulated immunity are the primary receptor complex, downstream signaling components, and transcriptional regulators. However, not much is known about negative regulators and their role in deactivated receptor signaling (Lee et al., 2020).

The goal of this research is to identify a mechanism for the regulation of RBOHD in Arabidopsis via phosphorylation and ubiquitination (Lee et al., 2020).

**Recent Progress**

Much of the recent progress regarding ROS production and RBOHD has already been conducted in the primary research article by Lee et al., 2020. Below are some other recent discoveries made by researchers in this field regarding possible mechanisms for ROS production.

Cytosolic glyoxylate reductase (GLYR1) may be another contributing factor to an increase in ROS production in Arabidopsis which is responsible for reducing glyoxylate to glycolate thus allowing reoxidation (Waszczak et al., 2018).

The gene BIK1 is also believed to play an important role in increased ROS production via phosphorylation and activation of Ca2+ channels which stimulate calcium-dependent protein kinases (CPKs) and result in RBOHD phosphorylation and increased ROS burst (Wang et al., 2020).

An increase in ROS can also occur as a result of MAMP activation, as opposed to PAMP, through the acquisition of calcium-dependent protein kinase 5 (CPK5) via phosphorylation and binding of Ca2+ to the N-terminal of RBOHD (Zhou and Zhang, 2020).

**Discussion**

Protein array technology was used to pinpoint a RING domain E3 ubiquitin ligase (PIRE) which was discovered to interact with PBL13 and the C-terminus of RBOHD (Lee et al., 2020). The same study found that PIRE was responsible for the ubiquitination of RBOHD, increased ROS production via PAMP, and, ultimately, decreased bacterial growth. The results of this research also indicate that PBL13 is negatively regulated and phosphorylates RBOHD resulting in ROS production in Arabidopsis. It was also found that PIRE undergoes phosphorylation upon the onset of the immune response (Lee et al., 2020).

flg22 (a bacterial PAMP flagellin) can induce ROS production, but mutations of certain serine residues in RBOHD can halt this production (Wang et al., 2020). In addition tocoupling with RBOHD, flg22 is also phosphorylated by PIRE (Lee et al., 2020).

Additional research suggests that RBOHD plays a role in monitoring stomatal opening and closing which may contribute to RBOHD’s ability to regulate the intensity of bacterial infection (Albert et al., 2020).

Certain research questions remain unanswered. One main question addresses the balance of positive and negative regulation in RBOHD’s N and C terminals in regard to post-translational changes pre- and post-infection (Wang et al., 2020). Another critical question asks whether the mechanism of ROS activation via RBOHs, RBOHD in Arabidopsis, can be applicable to other plants (Wang et al., 2020).

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**Primary manuscript:**

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