Mechanism of reactive oxygen species in winter Brassica napus growth and development and signal transduction under cold stress




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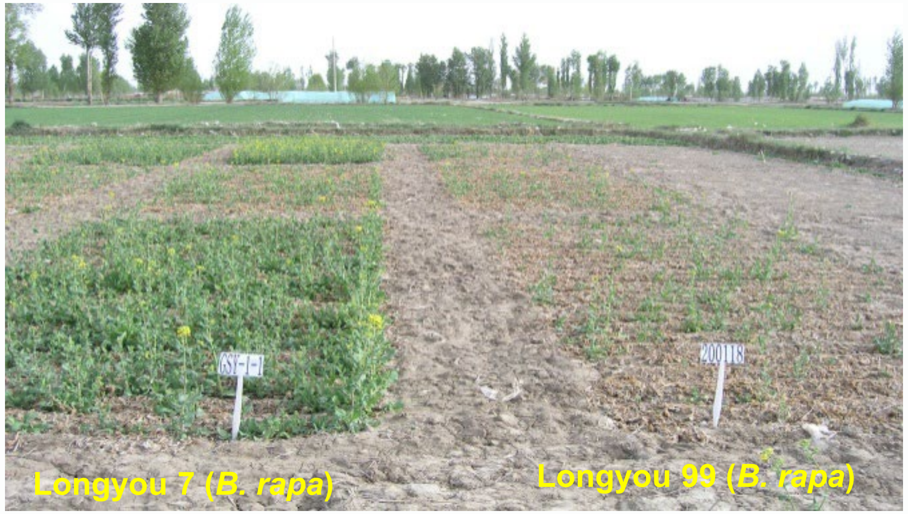
Contents

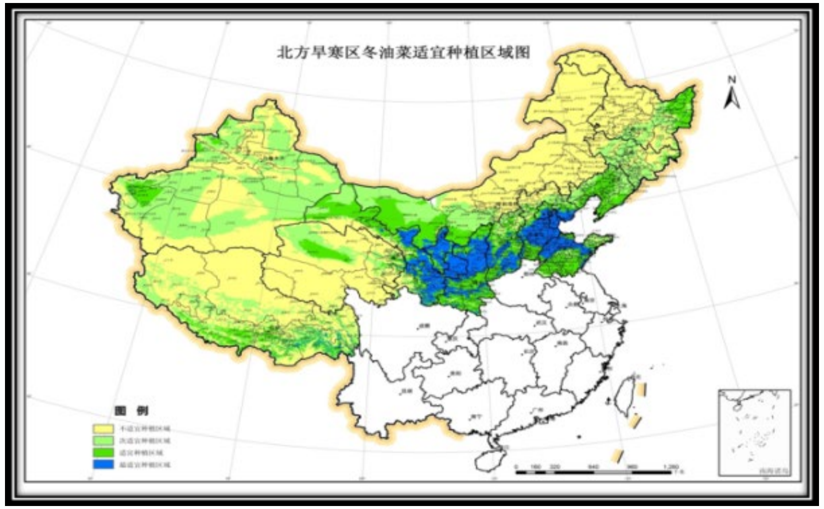
1. Purpose and significance

- Successful breeding and application of winter *B. rapa* varieties (Longyou 7,etc.), made winter rapeseed production become real in northern China (Sun et al. 2007)
- Strong cold-resistant winter rapeseed varieties have pushed Chinese winter rapeseed production northward to a range of 34°- 48° northern latitude



Longyou 7 overwintering at extreme low temperatures of -30 ℃

Performance of winter rape under 45 ºN condition



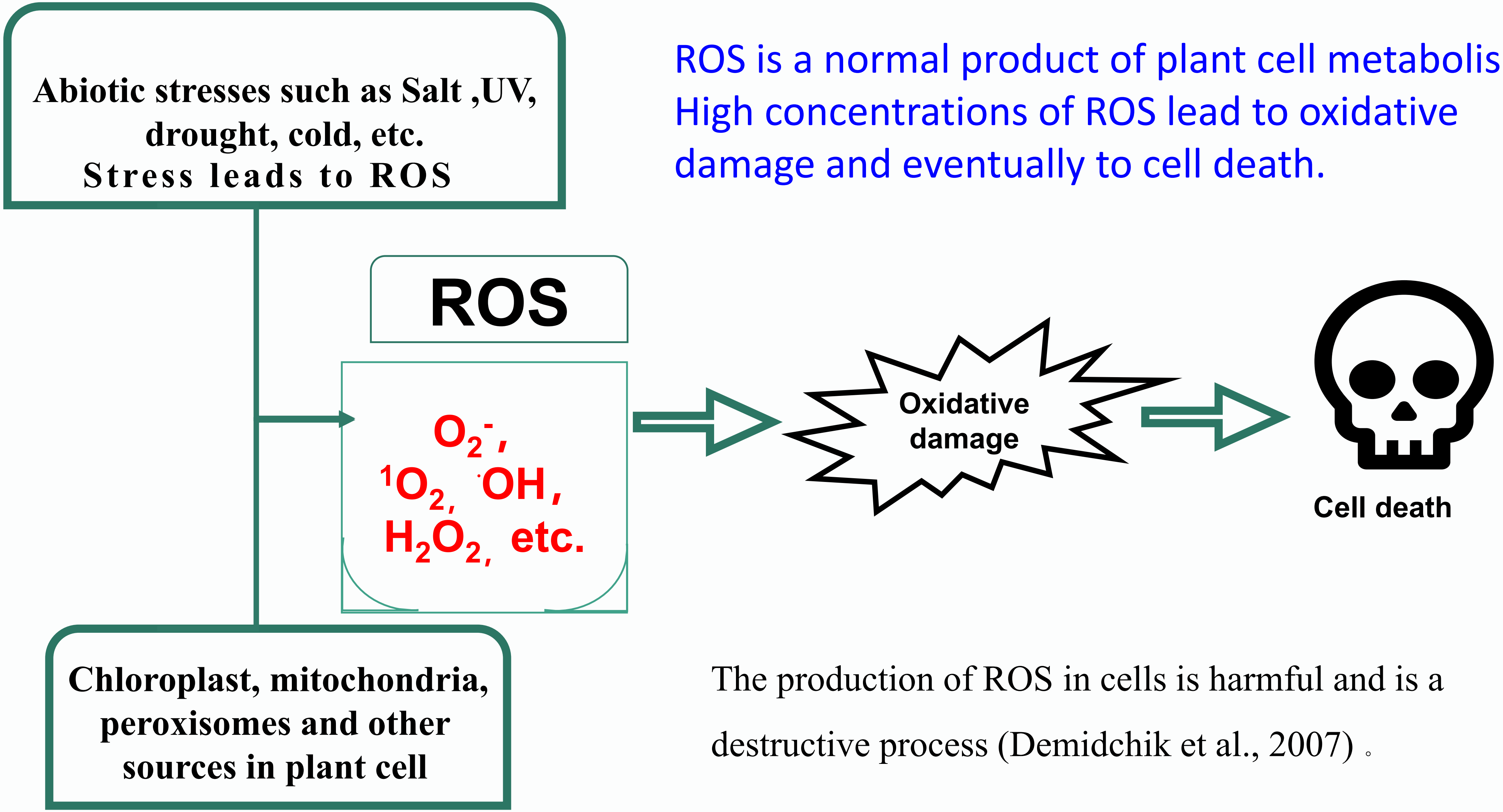
The new chinese rapeseed production map.Yellow & green area is the new chinese winter rapeseed production area, and white area is the traditional winter rapeseed production area

1. Purpose and significance

- *B. napus* has poor cold resistance and is difficult to overwinter in areas north of latitude 35 ° N.
- We used interspecific hybridization, backcross and continuous self-crossing methods of *B. napus* and *B. rapa* to create varieties with high quality and strong cold resistance.
- Resistant to low temperature of -24℃, The yield is higher than that of B.rapa, and the quality is excellent.
- It is of great economic and ecological benefit to increase the vegetation cover, windbreak and soil consolidation in northern winter and spring.



1. Purpose and significance

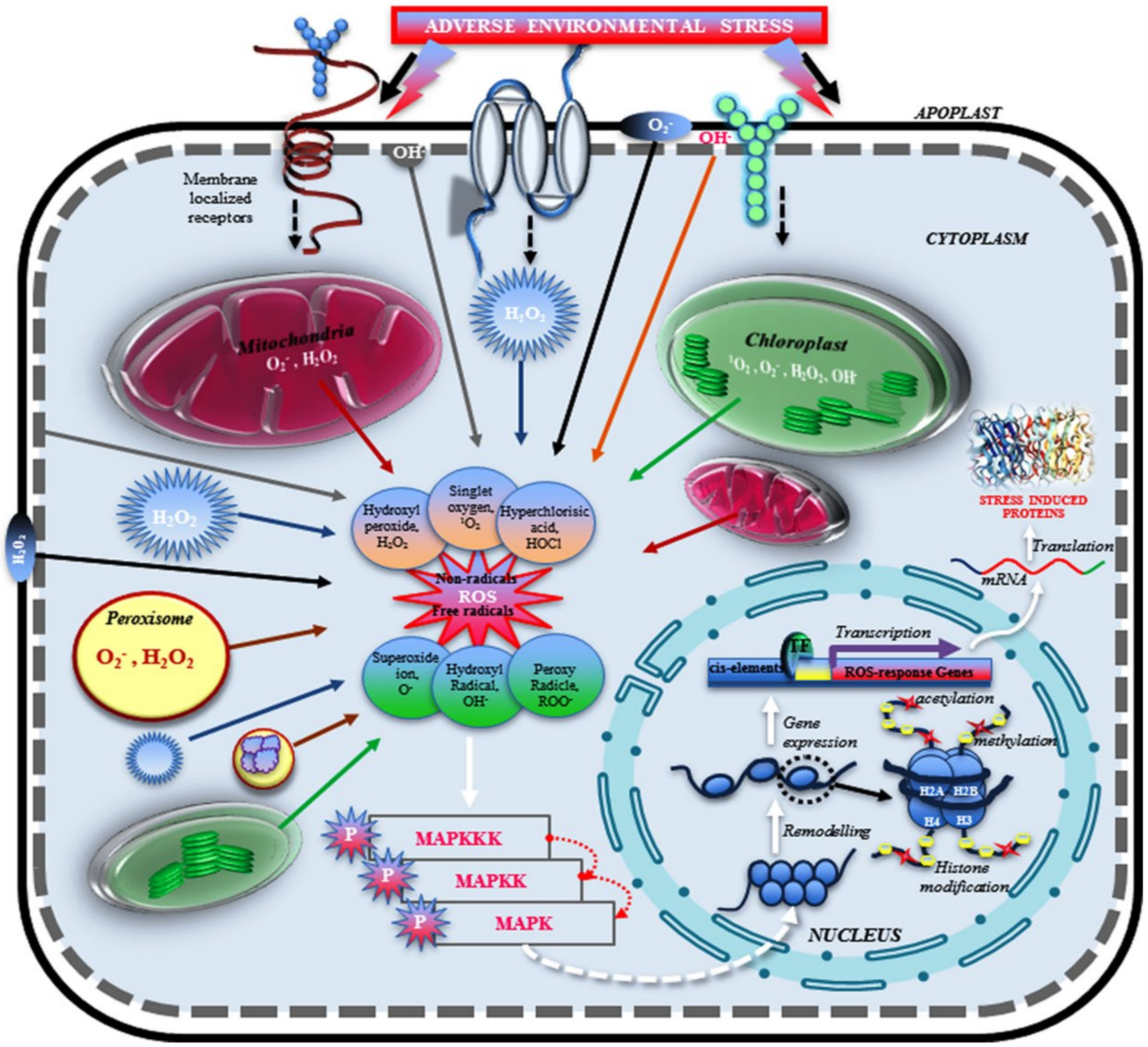


ROS is a normal product of plant cell metabolism. High concentrations of ROS lead to oxidative damage and eventually to cell death.

The production of ROS in cells is harmful and is a destructive process (Demidchik et al., 2007)。

1. Purpose and significance

- Despite ROS has destructive activity, it can act as a key signaling molecule in various cellular processes to activate second messengers, sense gene transcription, and alter enzyme activity to regulate physiological processes. (Marino et al., 2012).
- The ability of plants to clear ROS determines the type and intensity of plant cells' cold defense response and cold resistance (You and Chan, 2015).



A burst of plant NADPH oxidases. Marino et al., 2012

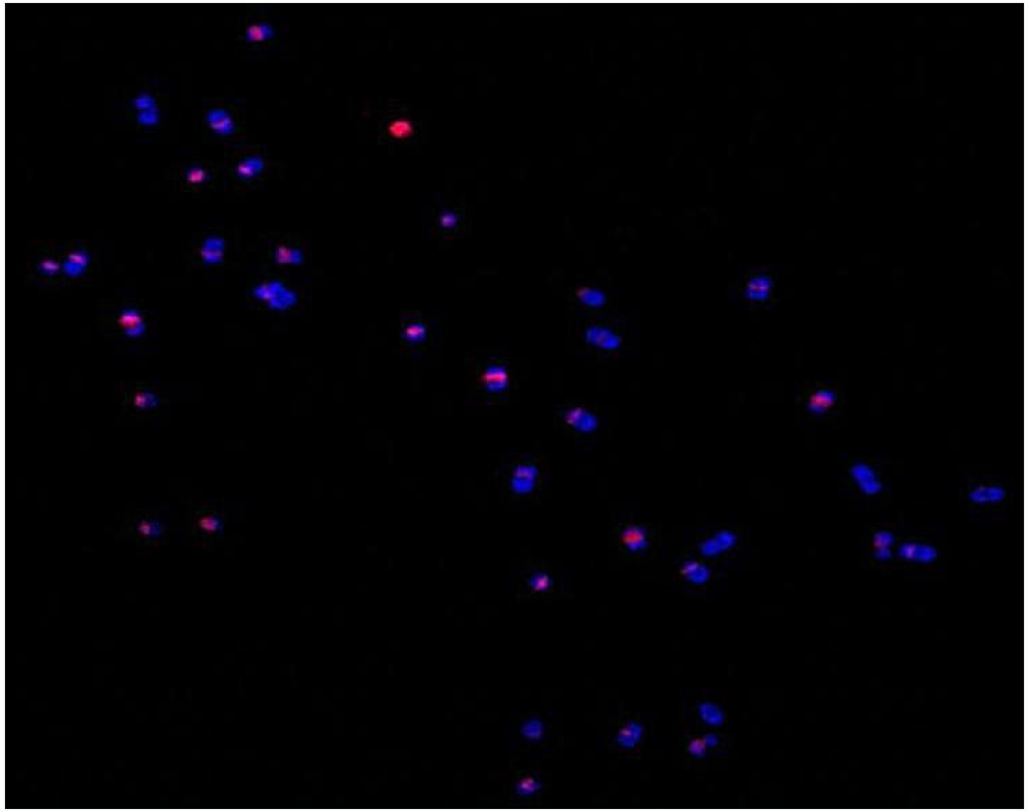
1. The purpose and significance

- In this study, strong cold resistant *B. napus* 16VHNTS309 and weak cold resistant Tianyou 2238 were used as research materials, starting from physiological, biochemical, cellular, molecular and other perspectives.
- Clarify the physiological metabolism, cellular signal transduction, and regulatory mechanisms of strong cold resistant winter *B. napus*. Explore the interaction between ROS and other signaling molecules to further explore cold resistance genes. Provide new insights for cultivating new varieties of cold resistant crops.

2. Analysis of Genetic Background in *B. napus* 16VHNTS309

Genetic background analysis:

- Using the maternal strong cold-resistant *B.rapa* Longyou 7 as A genome probe (red). The GISH results showed that A genomic signal was detected on 30 chromosomes of 16VHNTS309. The signal is mainly distributed in the middle centromere, satellite and short arm positions.
- It was speculated that there was an infiltration phenomenon of strong cold resistant *B.rapa* Longyou 7 A gene (small or large segmental gene) in the genetic background of the distant hybrid 16VHNTS309.



3. Physiological, biochemical and cytological analysis of cold resistance in strong cold-resistant winter *B. napus*

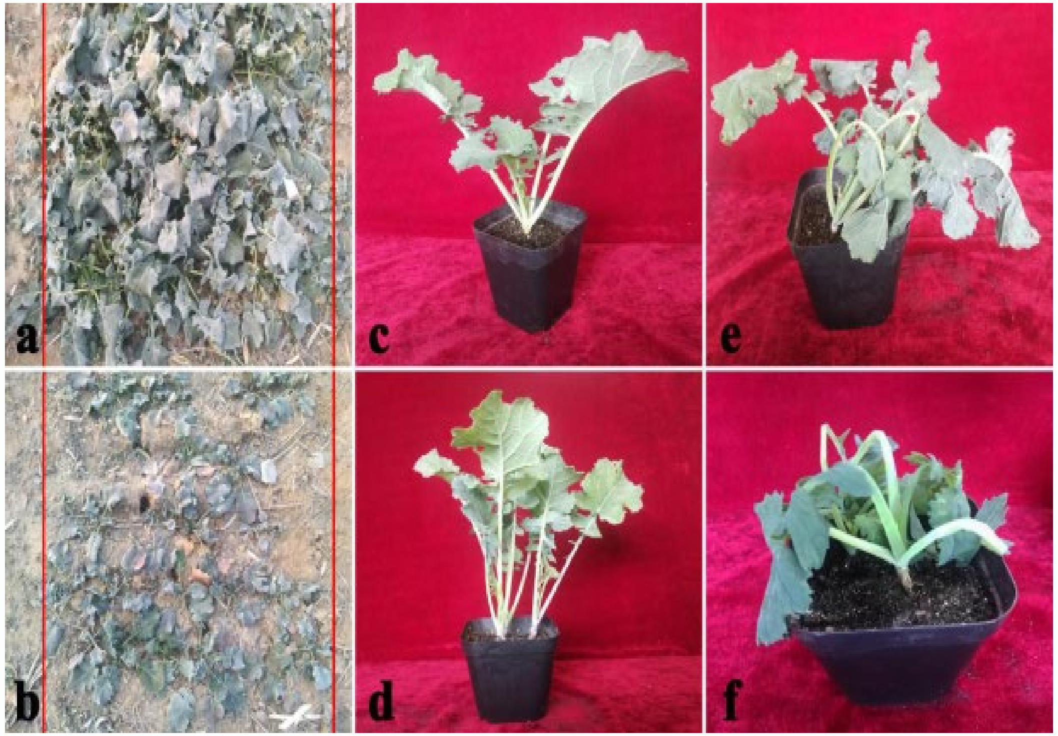
Morphological characteristics and physiological and biochemical index

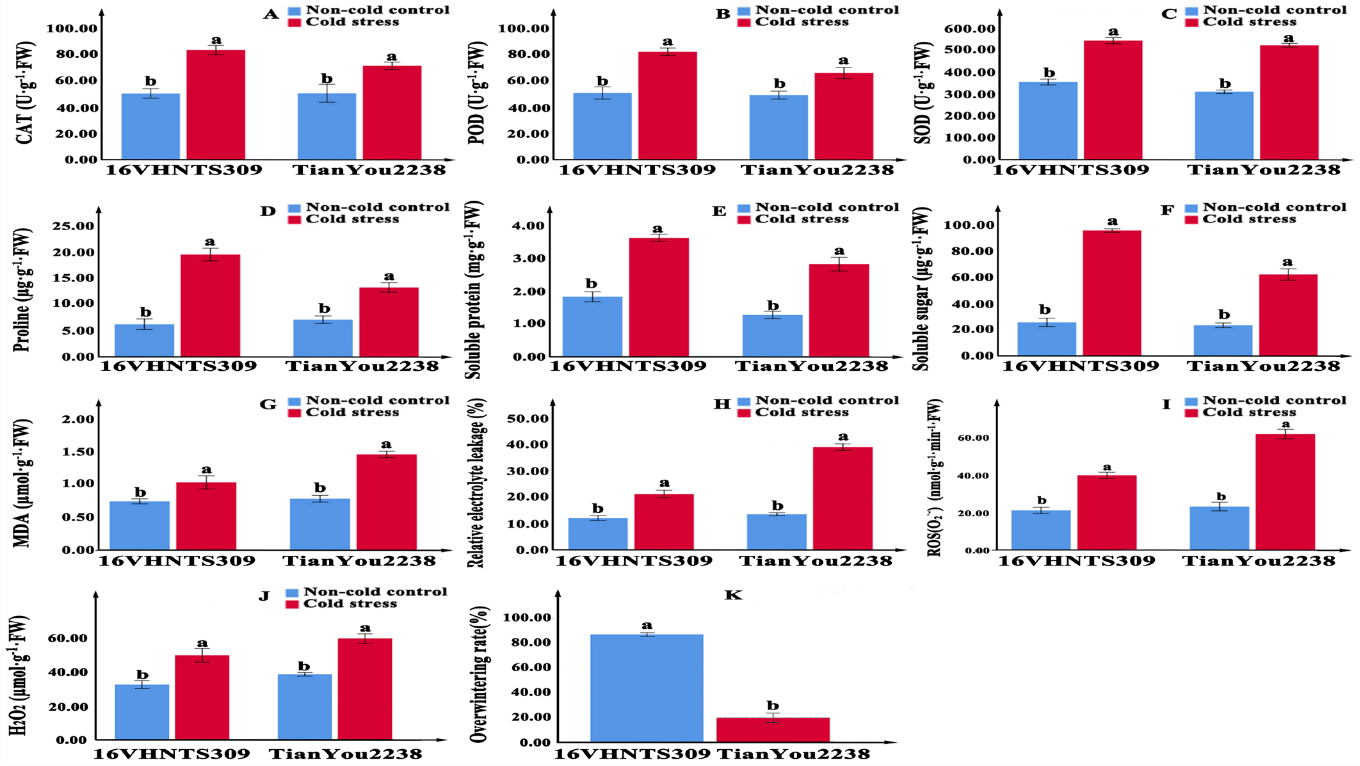
After low temperature stress, Tianyou 2238 showed obvious leaf wilting.

The activity of antioxidant enzymes (SOD, CAT and POD) and the accumulation of osmoregulatory substances (Pro, soluble protein and soluble sugar) of 16VHNTS309 were significantly higher than those of weak cold resistant Tianyou 2238, which effectively removed the accumulation of ROS in the body and reduced the damage to cells.

16VHNTS309

Tianyou 2238

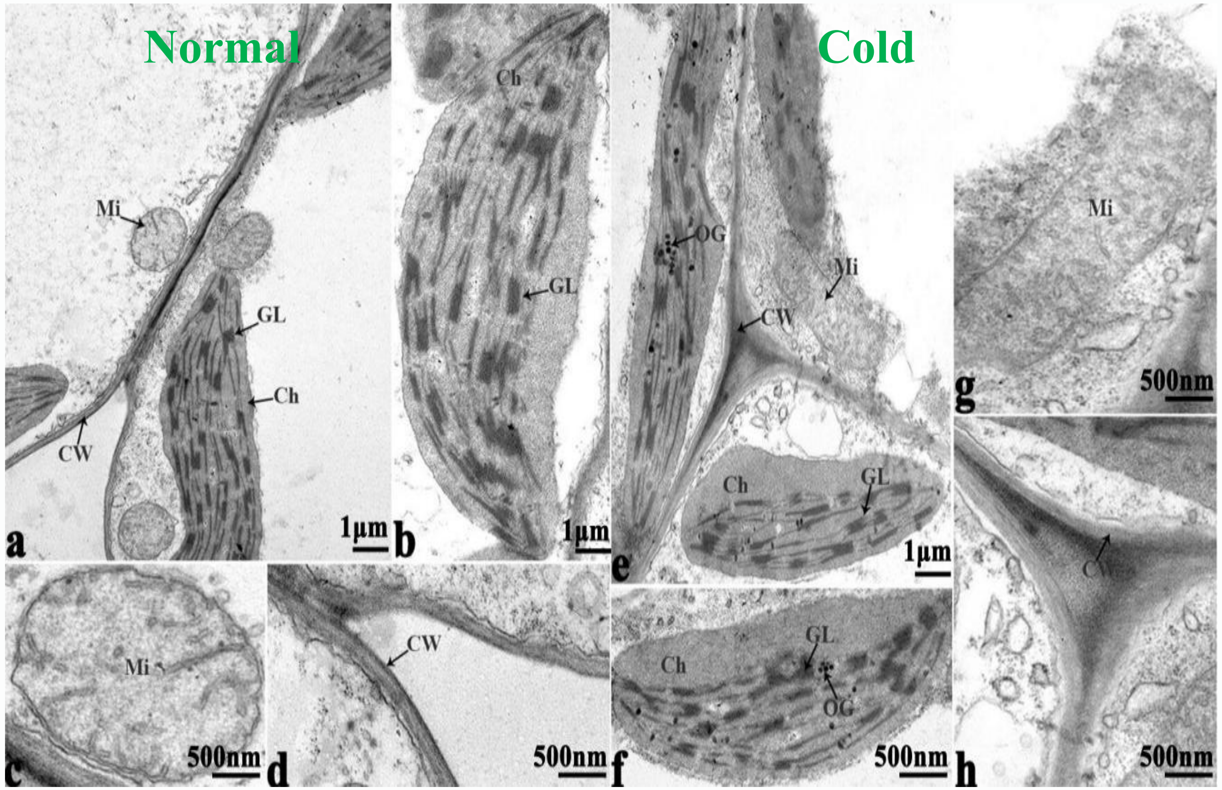




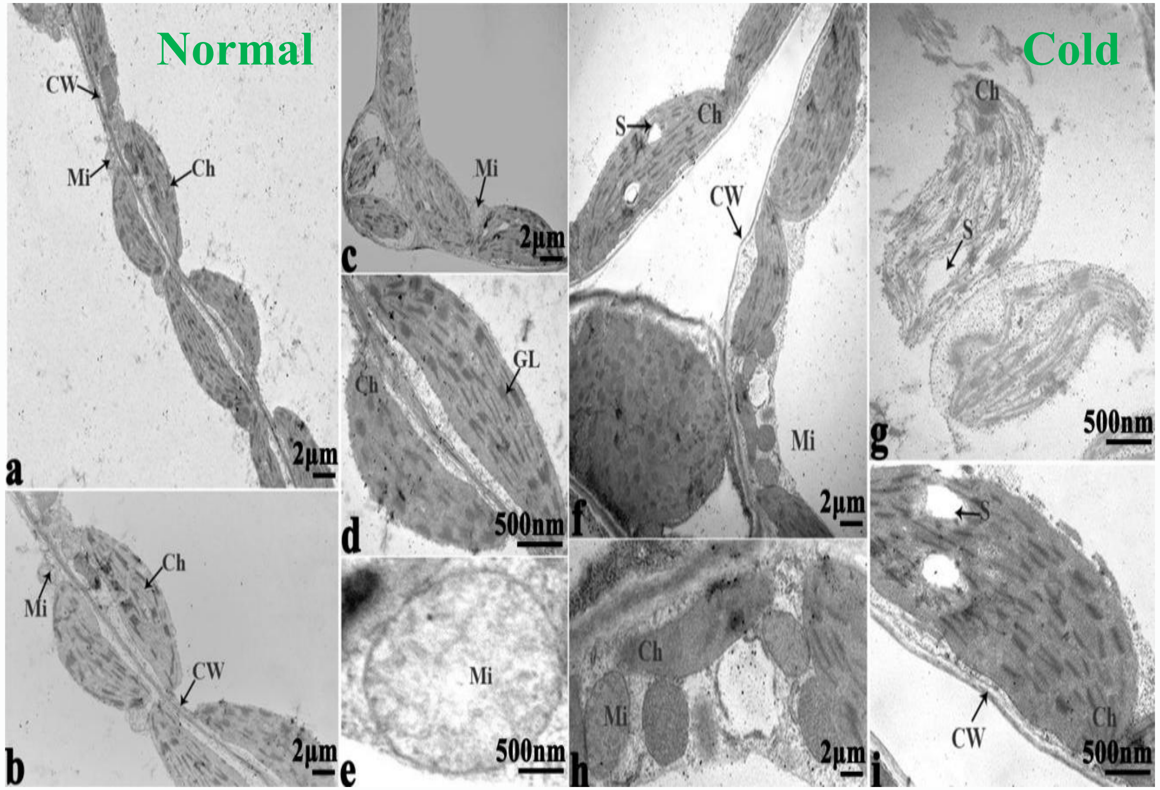
3. Physiological, biochemical and cytological analysis of cold resistance in strong cold-resistant winter *B. napus*

Ultrastructure observation of *B. napus* cells after cold stress treatment

16VHNTS309



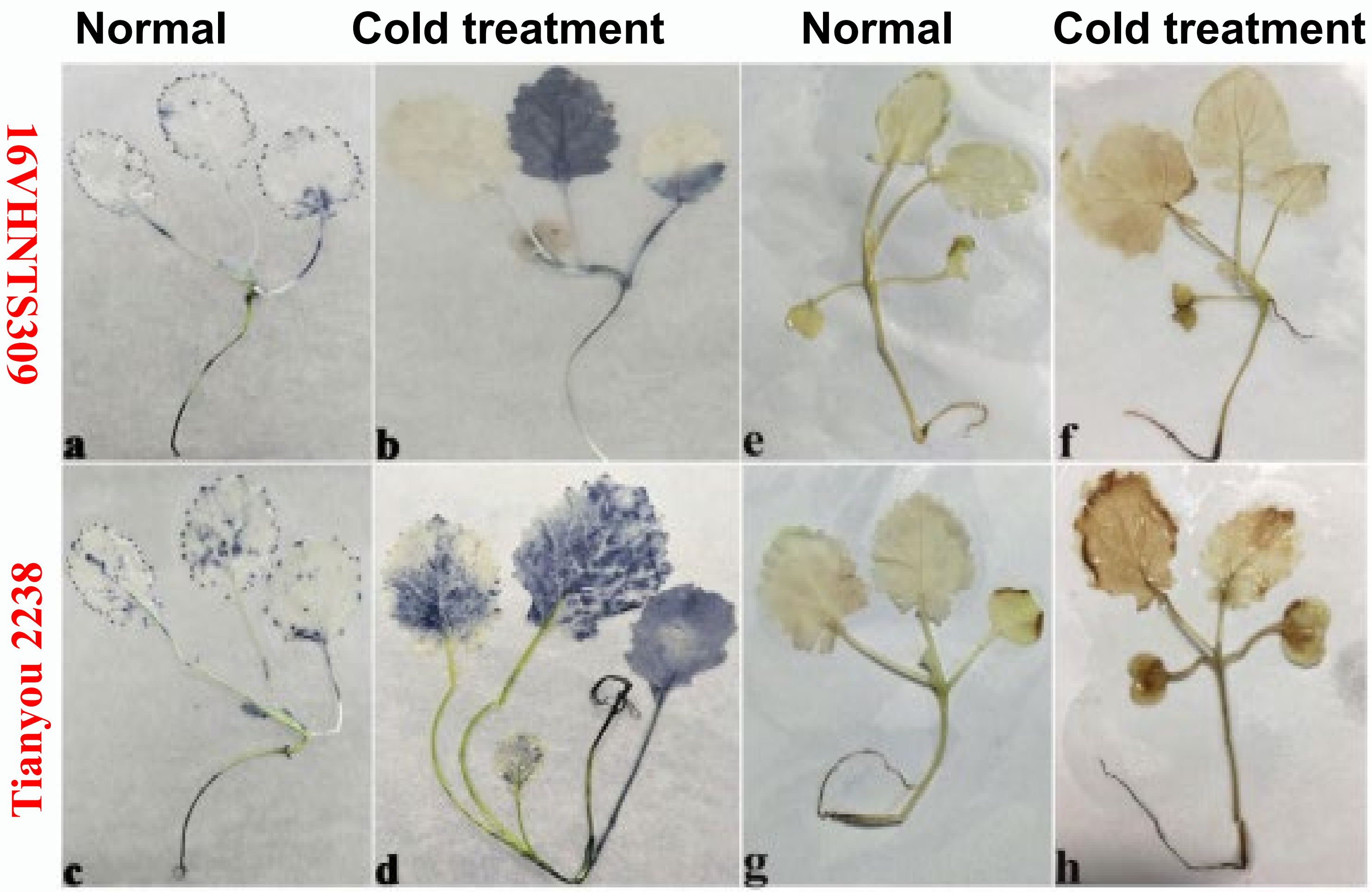
Tianyou 2238



After cold stress, the ultrastructure of the cells of Tianyou 2238 changed significantly (f, g, h, i), including cell wall rupture, cell membrane expansion, unclear contours and destruction of mitochondrial and chloroplast structures.

3. Physiological, biochemical and cytological analysis of cold resistance in strong cold-resistant winter *B. napus*

Qualitative analysis of H_2O_2 and O_2^-

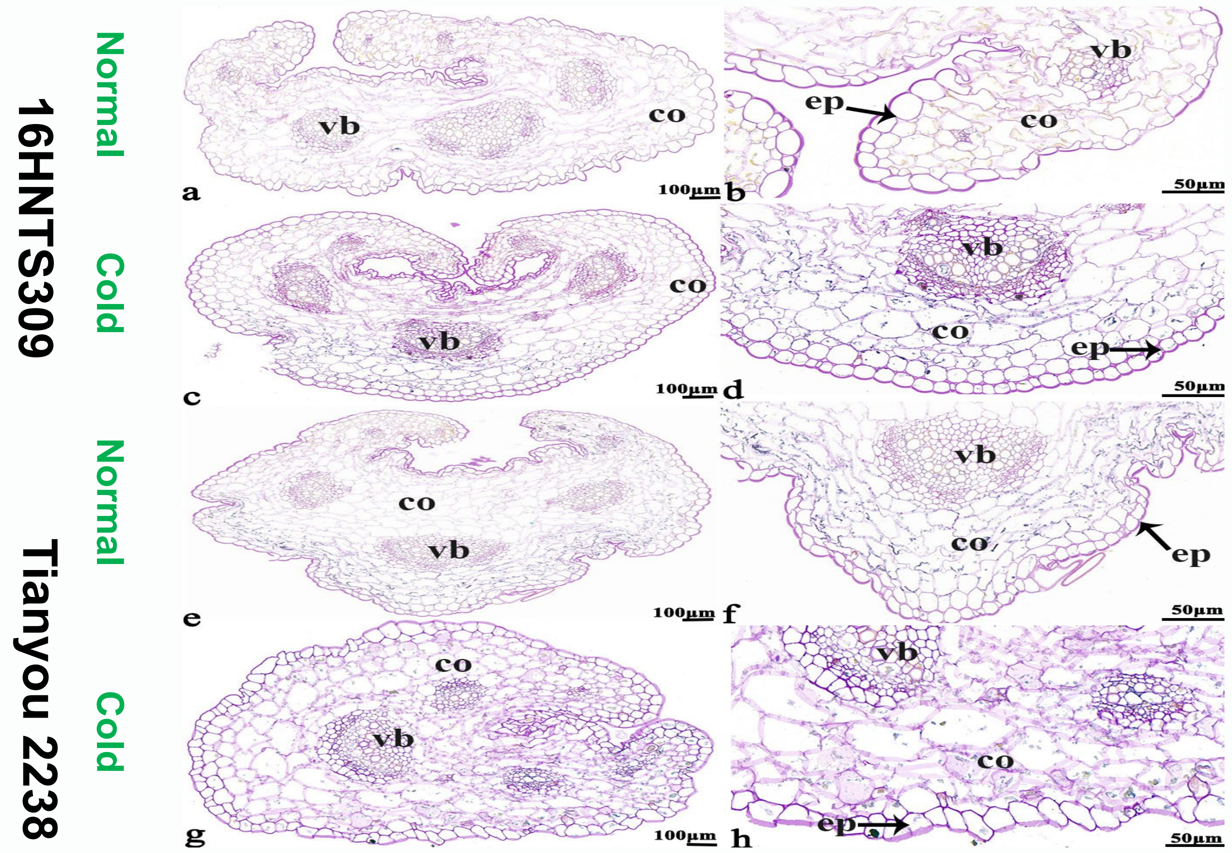


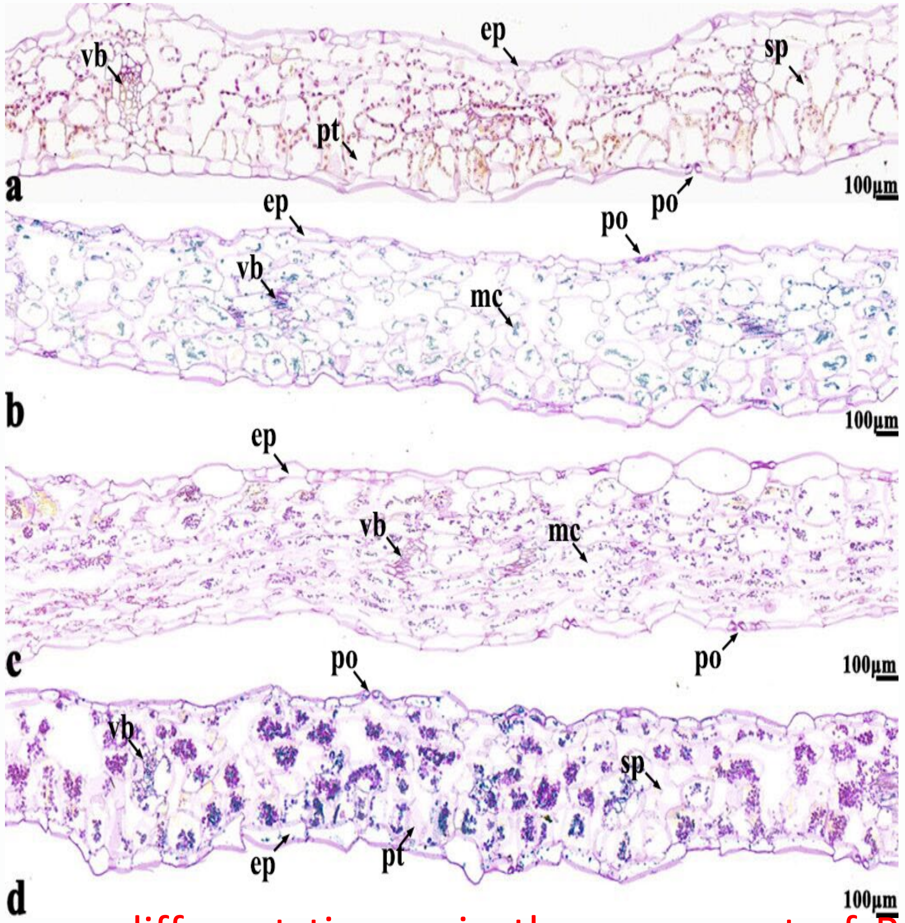
Blue polymerization (NBT) products representing O_2^- (a, b, c, d) and brown polymerization (DAB) products representing H_2O_2(e, f, g, h).

- After low temperature stress, ROS will be rapidly released, due to the difference in cold resistance of varieties, the accumulation of ROS (O_2^- and H_2O_2) in the cells of the strong cold resistance variety 16VHNTS309 was significantly less than that of the weak cold resistance Tianyou 2238.

3. Physiological, biochemical and cytological analysis of cold resistance in strong cold-resistant winter *B. napus*

Distribution of ROS (O_2^-) in tissue of *B. napus* after cold stress treatment





- It was found that ROS accumulation showed variability among different tissues in the same part of Brassica napus, and it was hypothesised that ROS in the chloroplasts mainly originated from chloroplasts.
- It is proposed that ascular bundle is a high-speed pathway for long-distance transport of ROS signals, and the synthesis, signal amplification, and systemic response expression of ROS signalling molecules further affects whole-plant resilience.

4. ROS involved in regulating the growth and development of strong cold resistant winter Brassica napus and cold stress signal response transmission

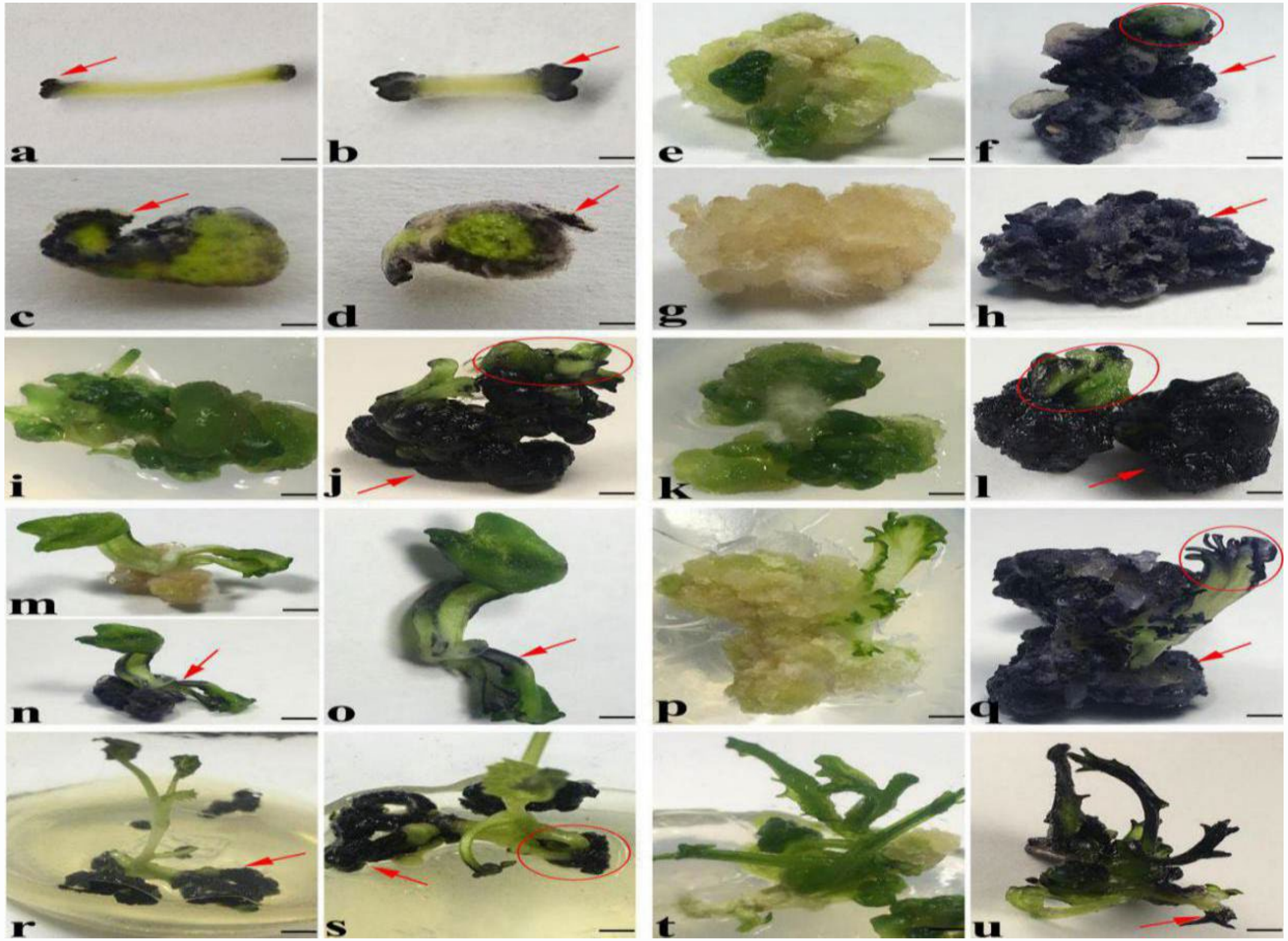
Accumulation rule of ROS (O_2^-) in callus of *B. napus*

Callus formation

shoot regeneration

middle stage

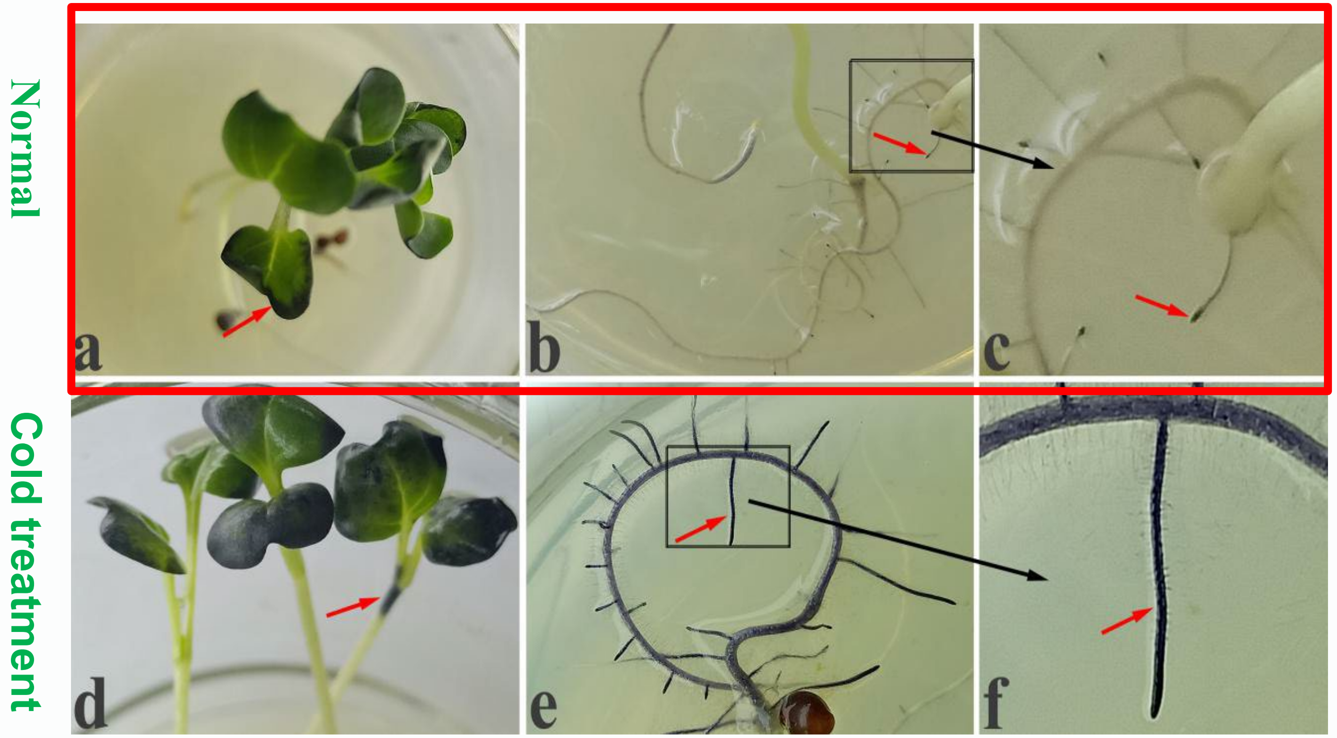
later period stage

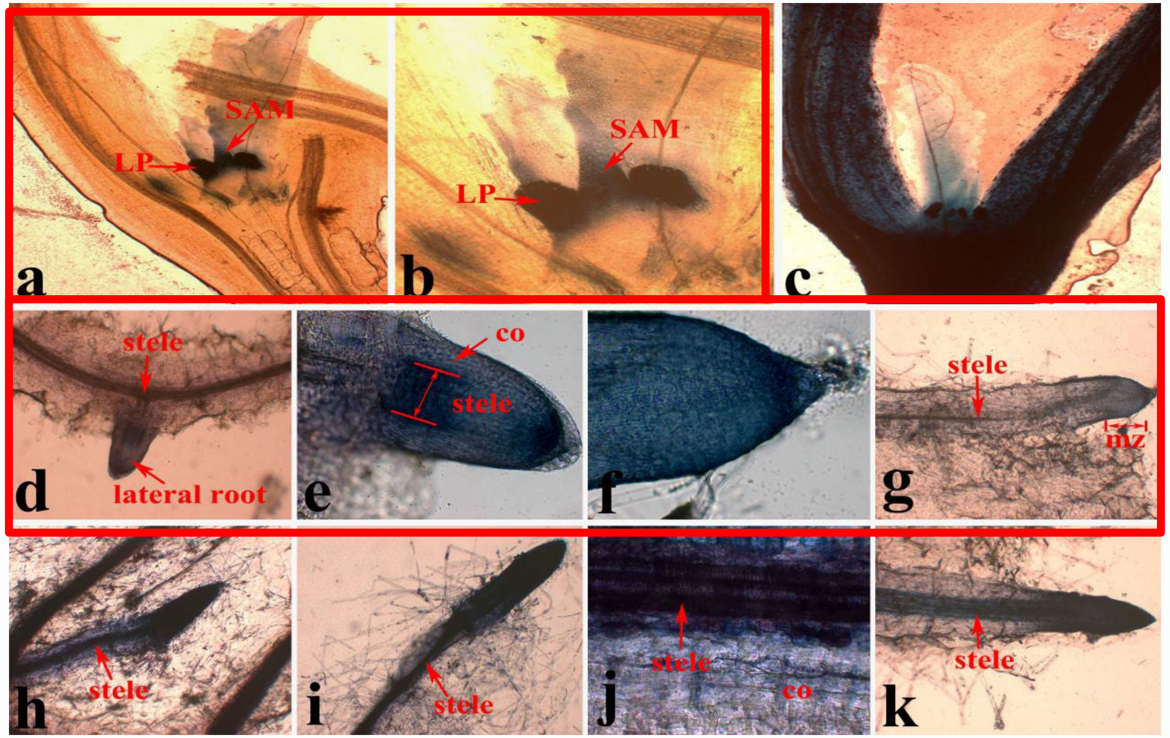


- 1) Callus cells, apical meristem cells, and leaf or stem edge tissue cells with the ability to divide detect a large number of O_2^- signals.
- 2) No O_2^- signal is detected around cells that have completed bud differentiation or have not formed calluses.
- 3) It was further confirmed that O_2^- plays an important role in the regulation of signaling in cell division.

4. ROS involved in regulating the growth and development of strong cold resistant winter *Brassica napus* and cold stress signal response transmission.

ROS (O_2^-) involved in the regulation of apical meristem cell division in *B. napus*





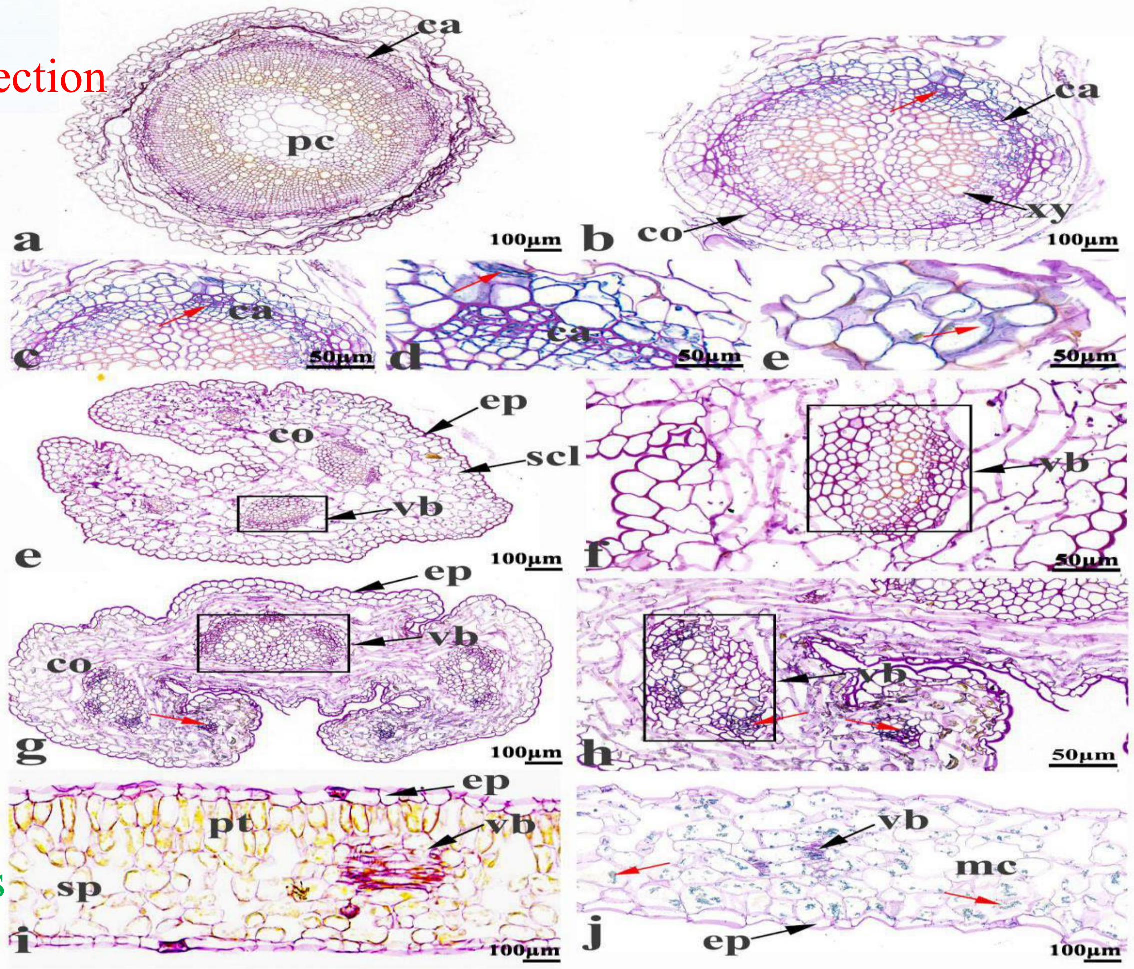
- Under normal conditions, O_2^- was mainly distributed in the root apex meristem, leaf edge, stem apical meristem area with strong cell division ability, indicating that the production of ROS is necessary for the normal growth and development of *B.napus*, and an appropriate amount of O_2^- plays a positive regulatory role in cell division.

4. ROS involved in regulating the growth and development of strong cold resistant winter *Brassica napus* and cold stress signal response transmission.

ROS (O_2^-) subcellular localization in cold resistant *B. napus* tissue cells

cross section

roots



stem
s

leaves

O_2^- subcellular localization: O_2^- signaling was detected in the root (c, d, e), stem (g, h) and leaf (figure j) vascular bundle tissue of *B.napus*.

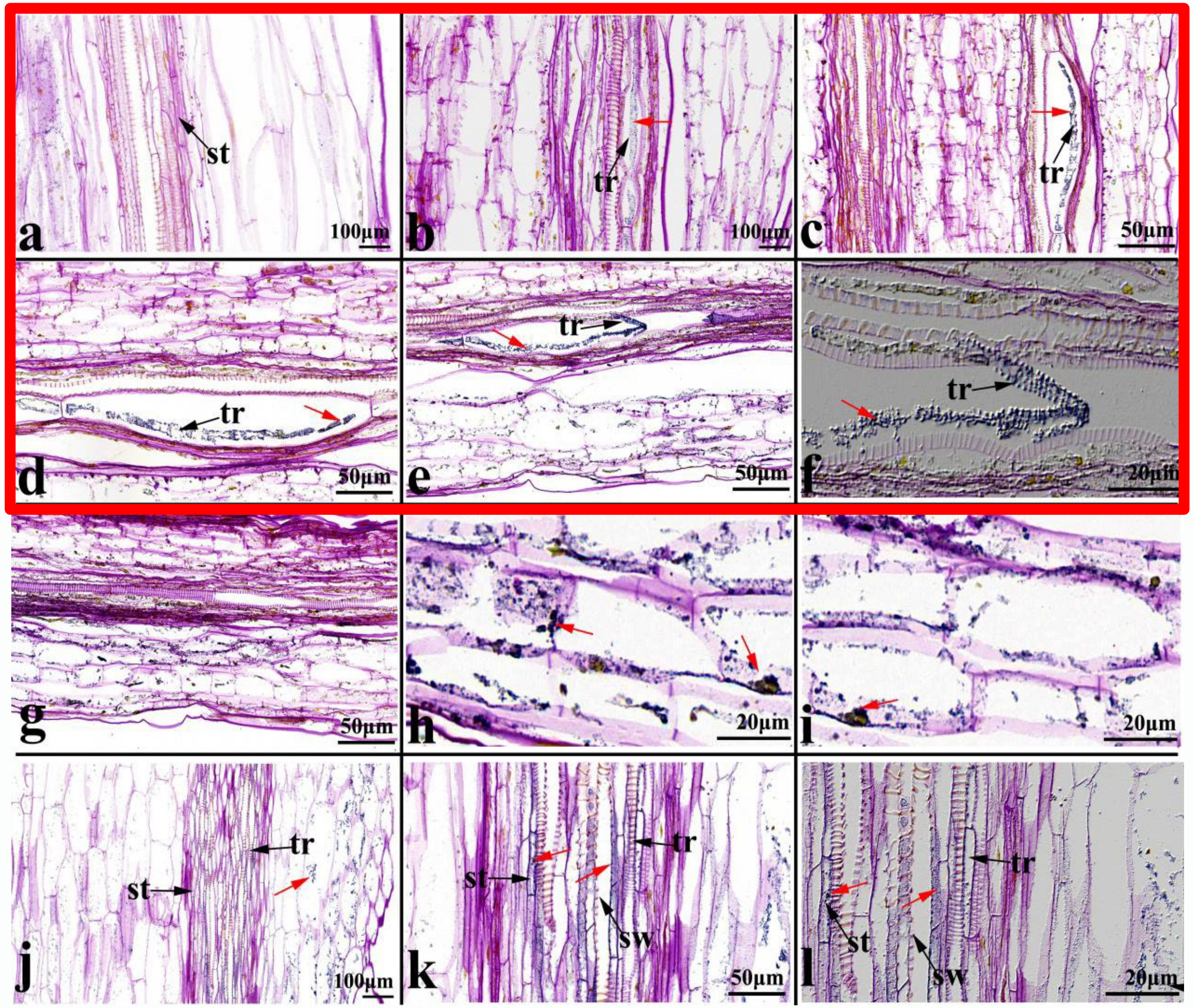
- After cold stress (4℃) treatment for 48 h: The accumulation of O_2^- in vascular tissue of roots, stems, and leaves is increased and significantly more than normal treatment.
- This further suggests that the ROS accumulated by vascular bundle cells of strong cold resistant *B.napus* after cold stress may be related to signal response expression.

4. ROS involved in regulating the growth and development of strong cold resistant winter *Brassica napus* and cold stress signal response transmission.

ROS (O_2^-) subcellular localization in cold resistant *B. napus* tissue cells

stem

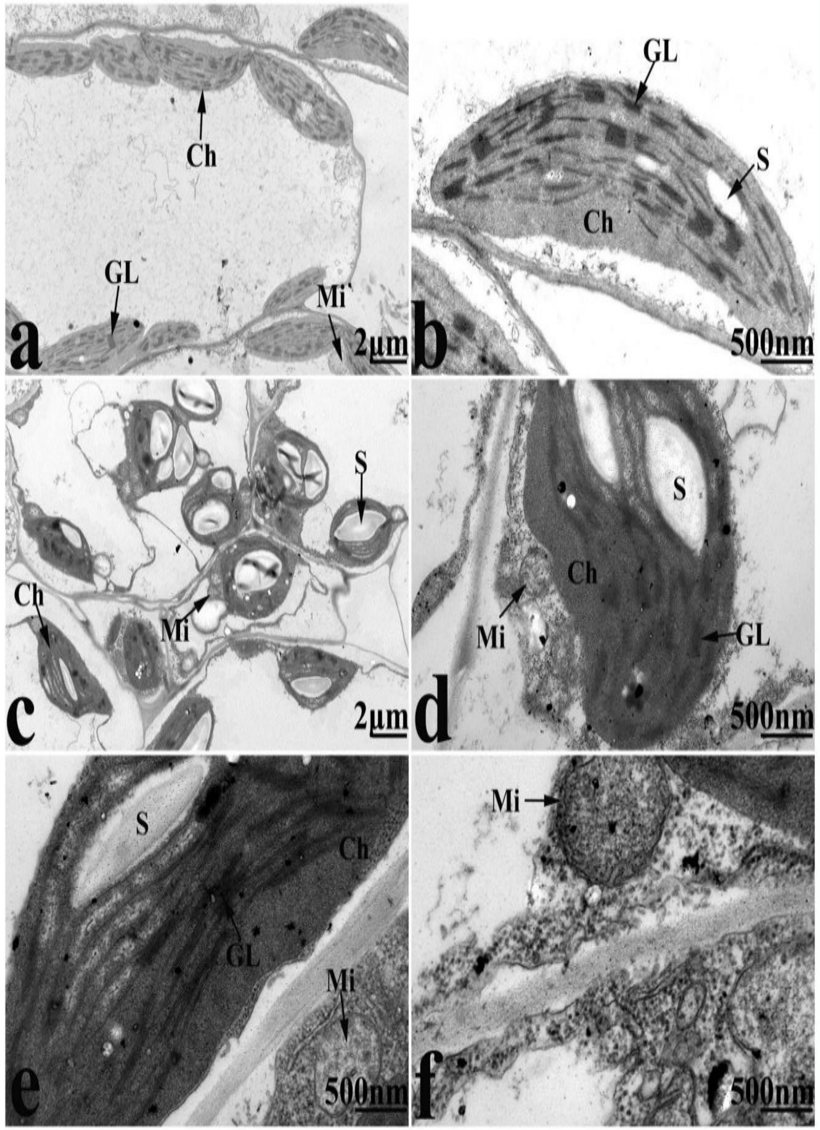
leaves

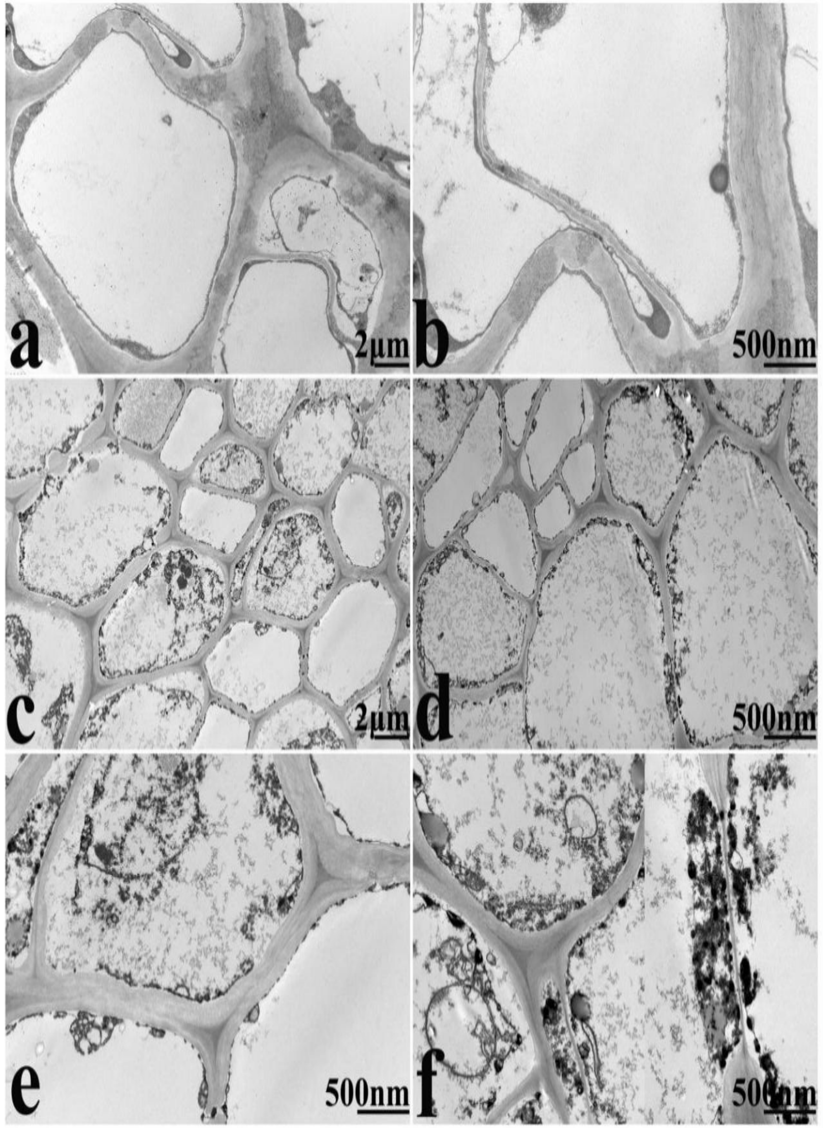


- After cold stress, O_2^- signaling could be detected by sieve tubes(st), plasma membrane adnexal regions and ductal cells of *B.napus* leaf veins and petiole stems.
- The sieve tube and catheter of vascular bundle tissue may be the fast channel of ROS signal transmission

4. ROS involved in regulating the growth and development of strong cold resistant winter *Brassica napus* and cold stress signal response transmission.

Ultrastructural localization of ROS (O_2^-) signal in strong cold resistant *B. napus* tissue cells

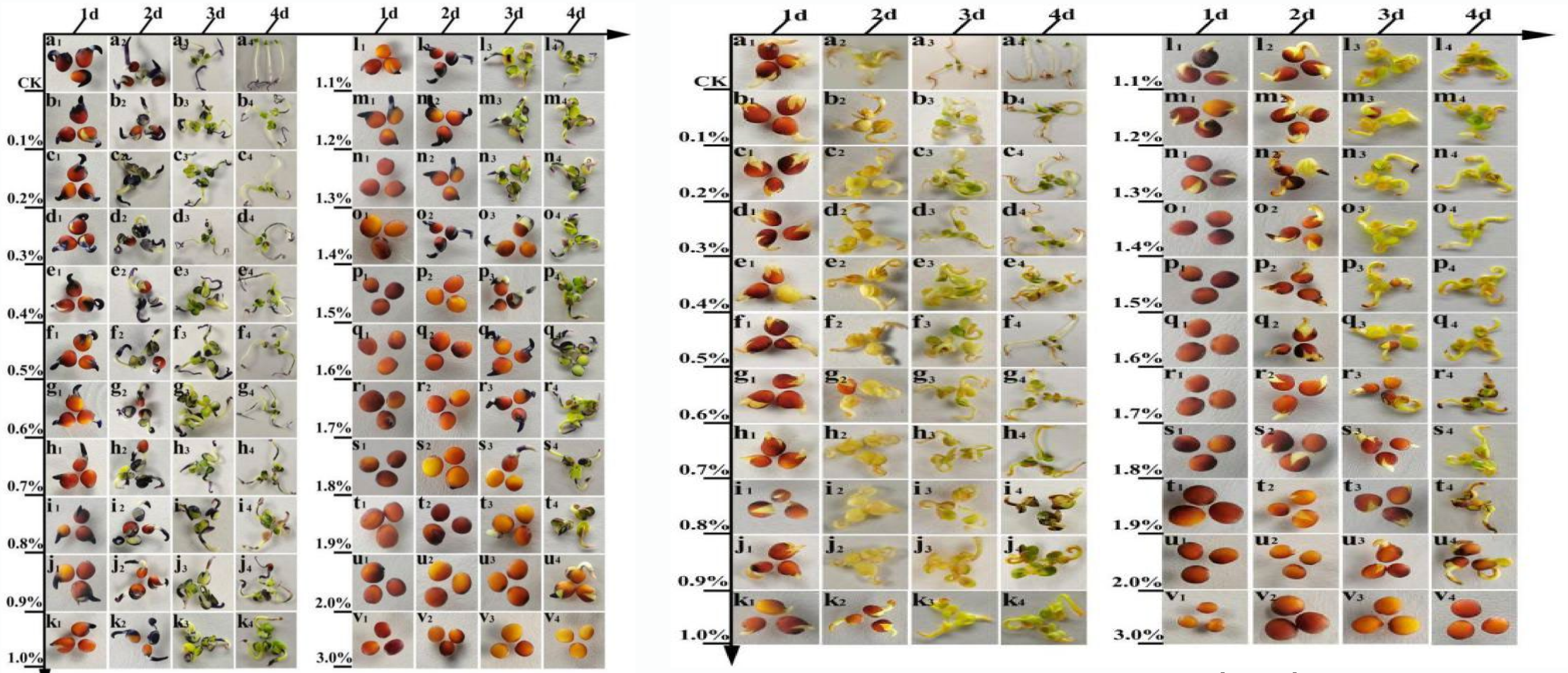




Cold+NBT(c, d, e,f)	
leaf	root
chloroplast	
mitochondria	mitochondria
Plasma membrane NADPH	Plasma membrane NADPH

- After cold stress, plants under go a large outbreak of ROS.
- Chloroplasts, mitochondria and plasma membrane NADPH oxidase are involved in ROS formation, but there are differences in the mechanisms of ROS production by cells of root and leaf tissues

5. Analysis of ROS concentration threshold range and dynamic equilibrium regulation mechanism of ROS in strong winter B. napus



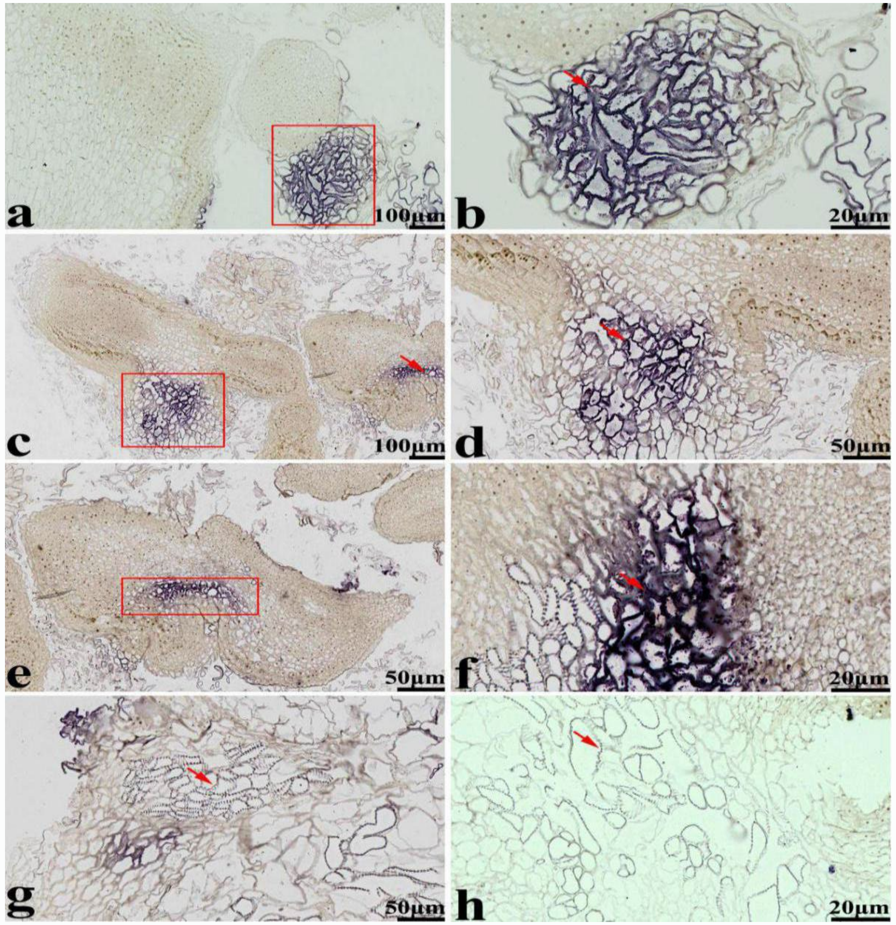
NBT staining (O_2^-) DAB staining (H_2O_2)

- The results show that a large amount of O_2^- and less H_2O_2 accumulate at the mesocotyl tip during the germination of *B.napus* seeds.

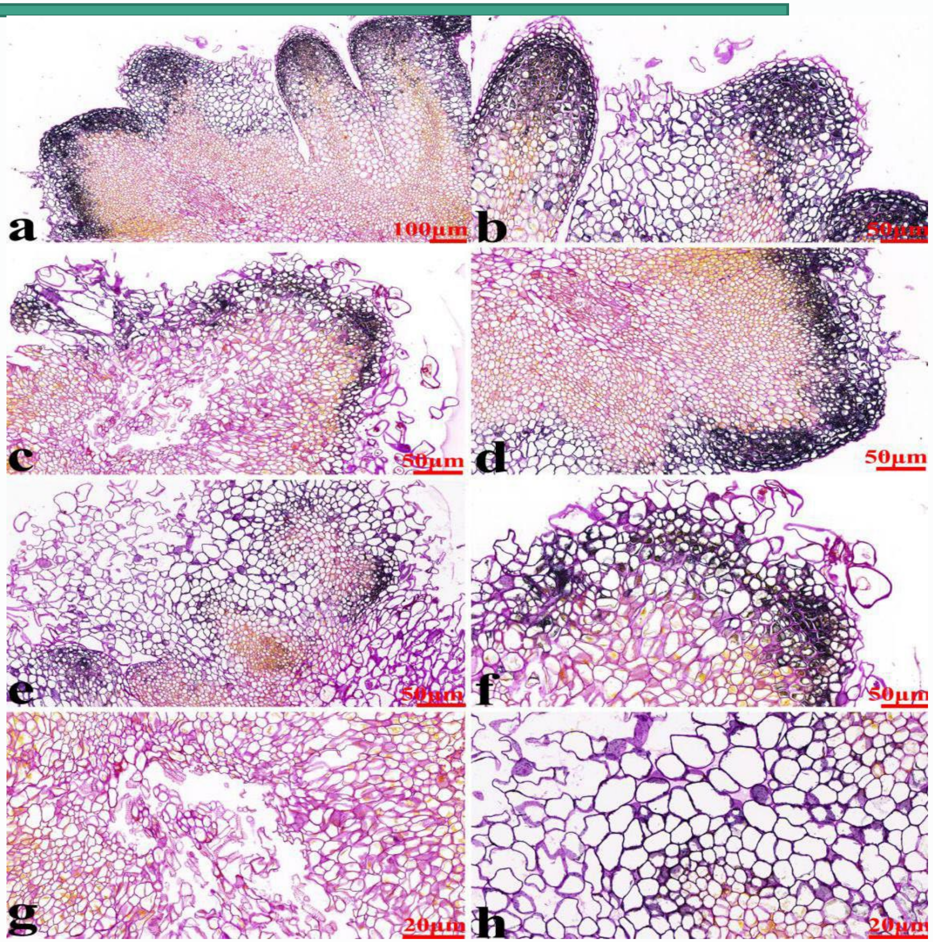
5. Analysis of ROS concentration threshold range and dynamic equilibrium regulation mechanism of ROS in strong winter B. napus

- Higher concentration of H_2O_2 (1.0% - 3.0%) was sprayed on the normally growing seedlings of *B.napus* 16VHNTS309 ,
- the results showed that with the increase of H_2O_2 concentration, the leaves gradually turned yellow. When the concentration is greater than 2.1%, a large area of leaf death occurs.

5. Analysis of ROS concentration threshold range and dynamic equilibrium regulation mechanism of ROS in strong winter B. napus



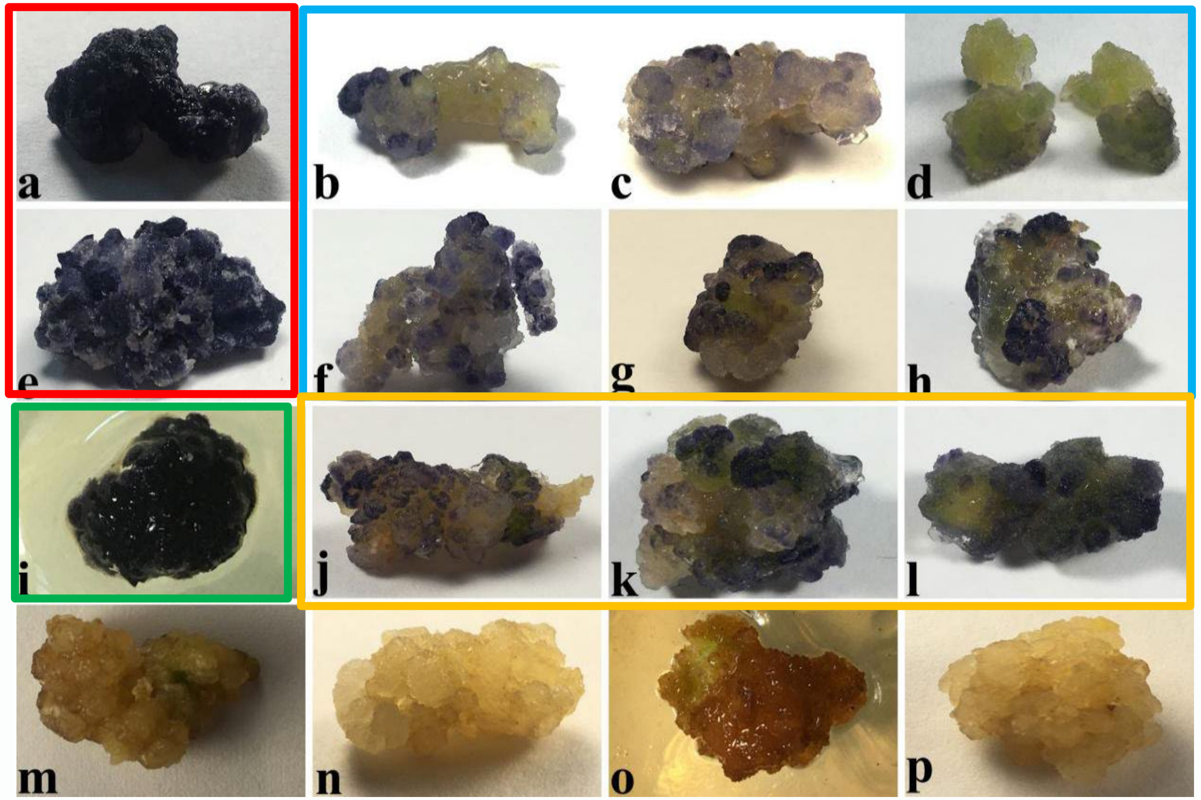
BnUPB1 subcellular localization

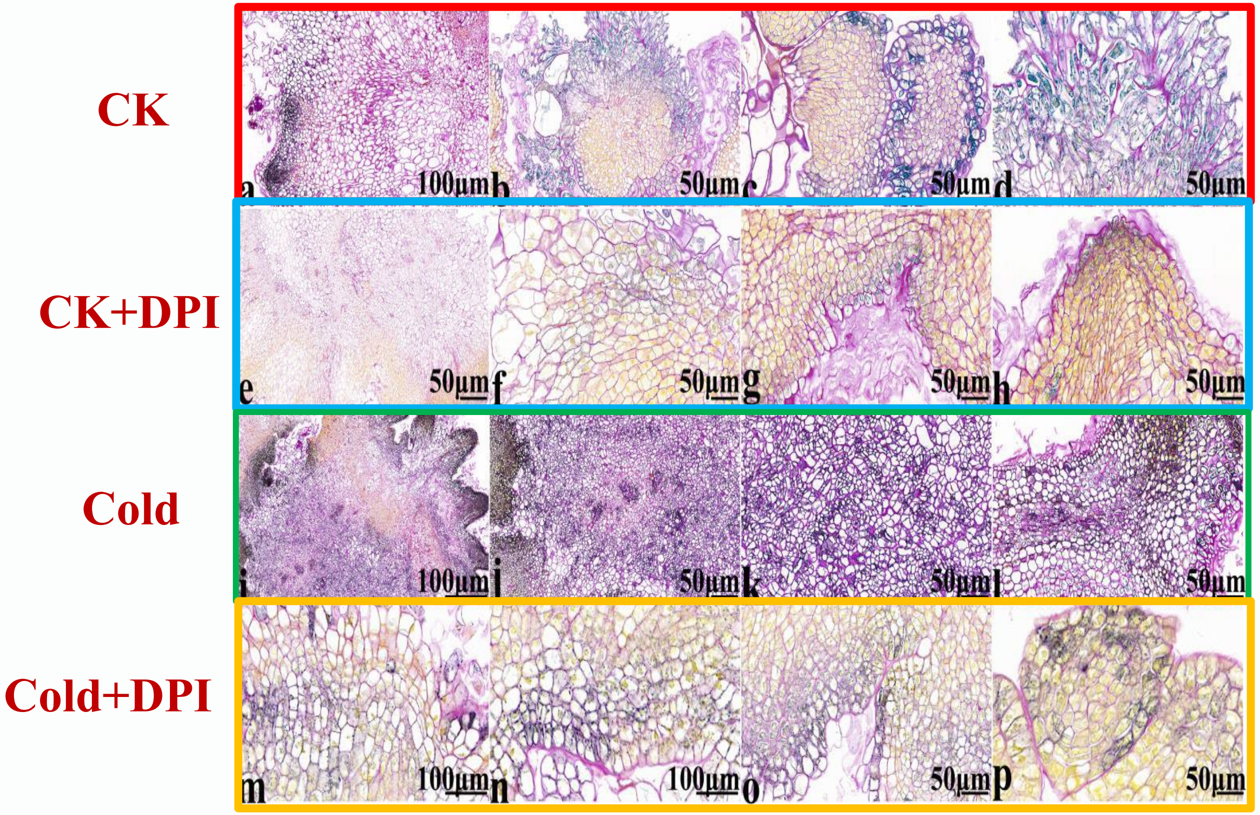


O_2^- subcellular localization

- A large amount of O_2^- accumulates in the meristem region of *B.napus* cells and the apical meristem region, but no UPB1 expression signal is detected, which further supports that silent expression of *UPB1* gene regulates the accumulation of O_2^-, thereby enhancing cell division ability.

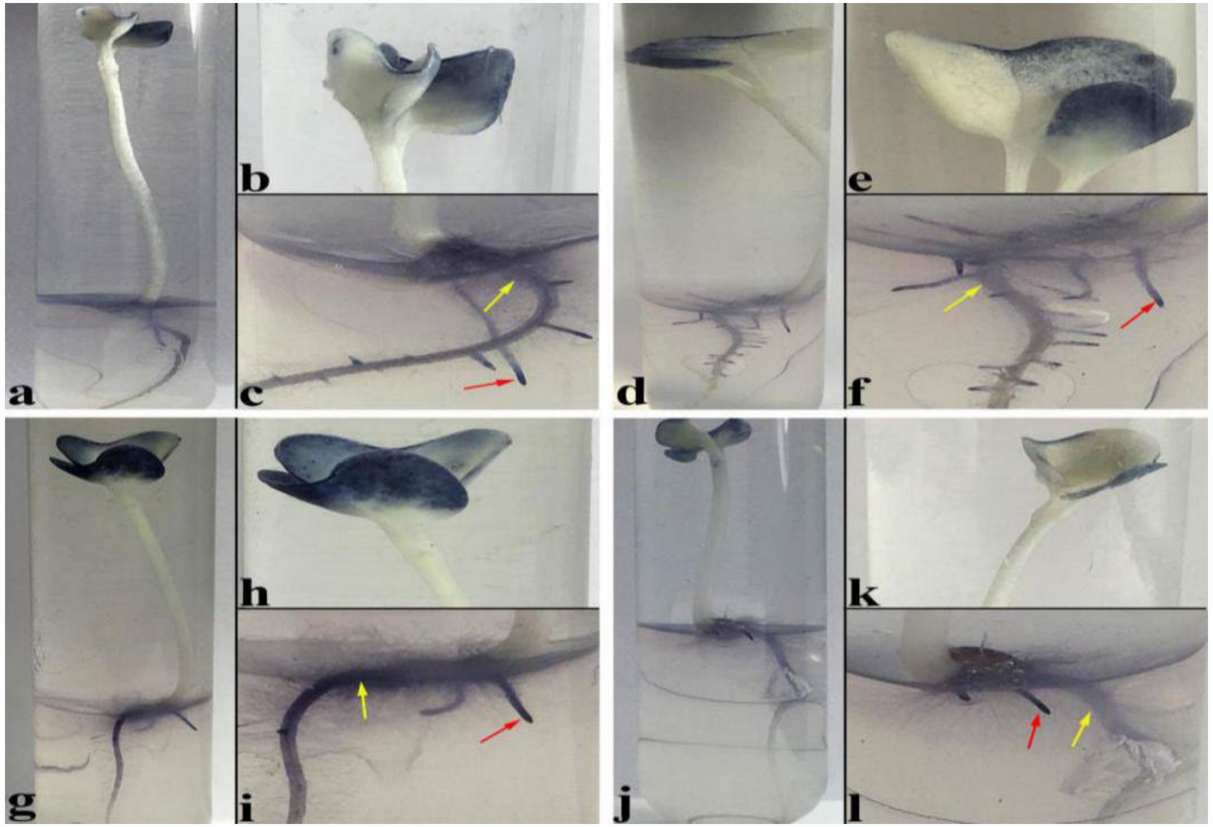
6. External application of DPI verified the role of NADPH enzyme in the growth and development of strong cold resistant *Brassica napus* and cold stress signaling



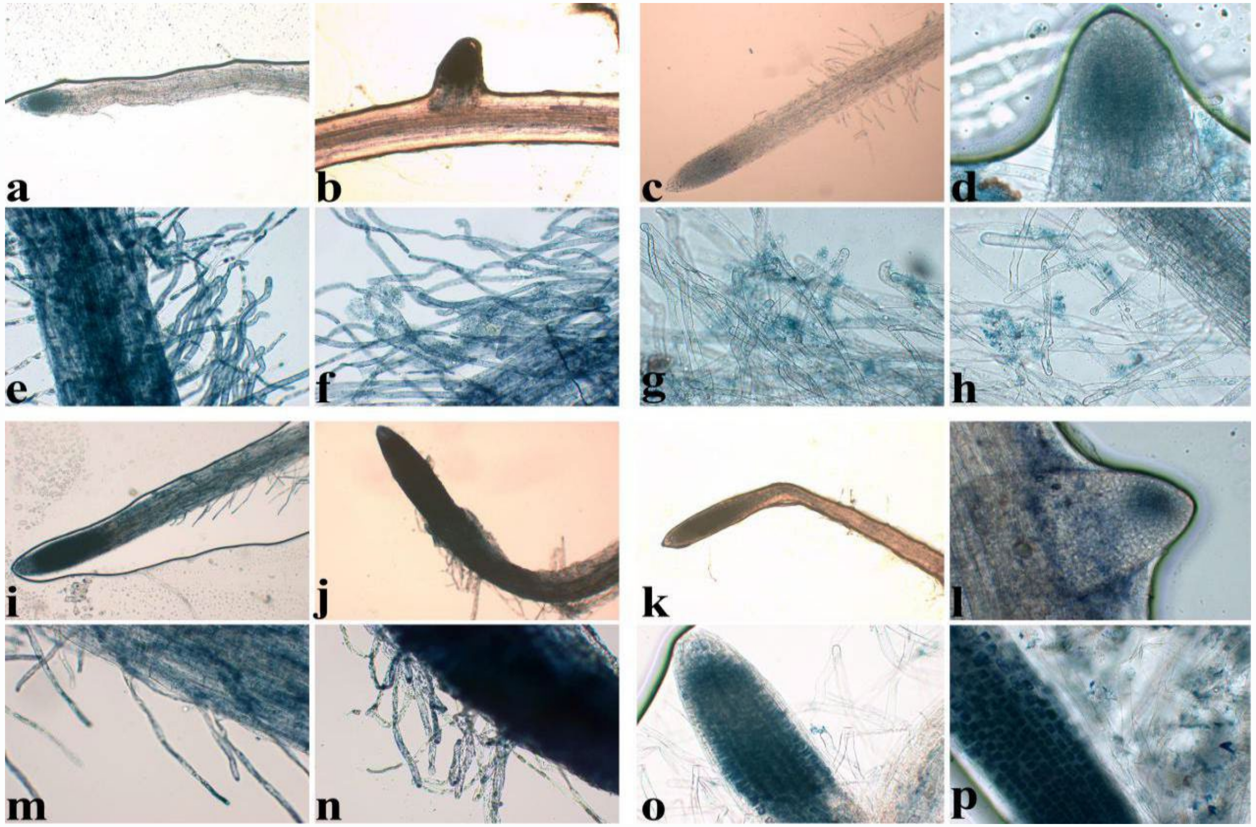


- The results of O_2^- subcellular localization showed that O_2^- was mainly distributed in the plasma membrane region. After DPI treatment, no O_2^- signal was detected in the plasma membrane region of cells
- It is further shown that NADPH enzyme-mediated ROS plays a major role in cold stress signaling, but there are still other ROS generation mechanisms that work synergistically.

6. External application of DPI verified the role of NADPH enzyme in the growth and development of strong cold resistant *Brassica napus* and cold stress signaling



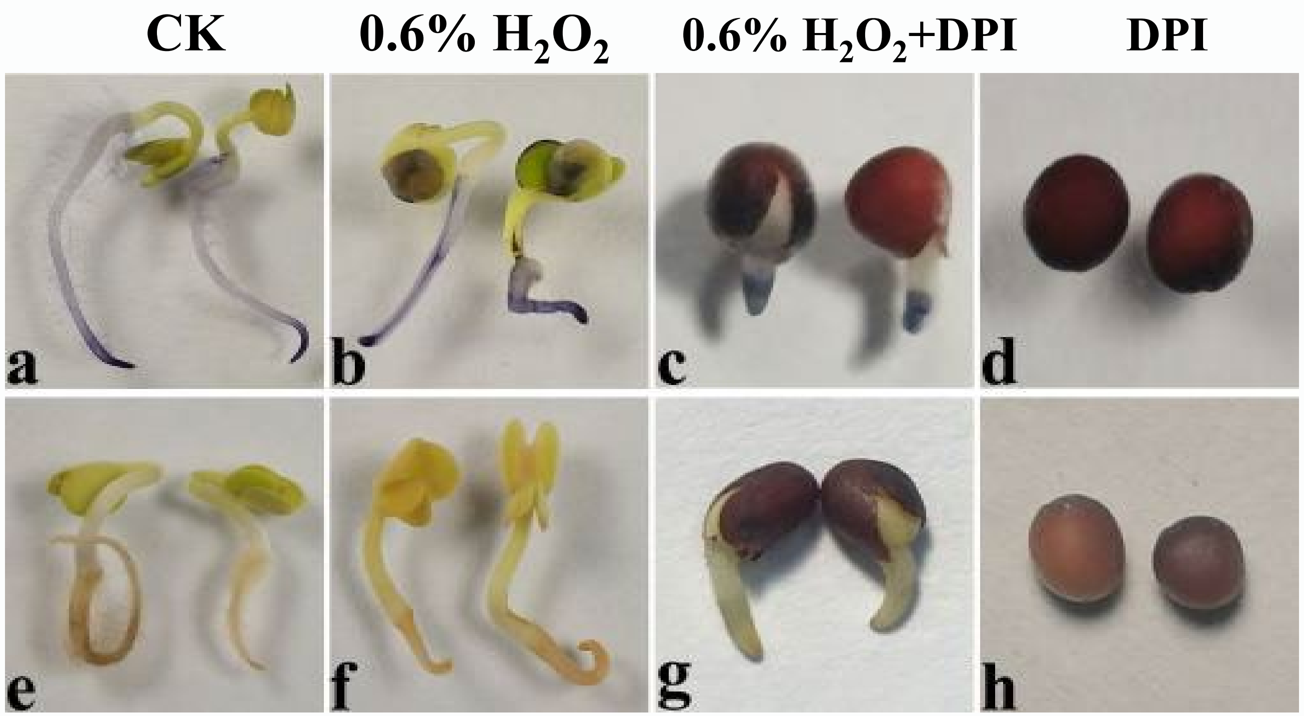
Cold Cold+DPI



Cold Cold+DPI

- After 24h treatment with CK (25℃), CK +DPI, 4 ℃ and 4 ℃+DPI, O_2^- mainly distributed in the meristem regions of leaf edges and root tips, and the accumulation of O_2^- was significantly reduced after DPI treatment.
- NADPH enzymes are one of the main sources for apical meristem cells, but it is hypothesised that ROS produced by mitochondria play a key role in the region of apical meristem cells.

6. External application of DPI verified the role of NADPH enzyme in the growth and development of strong cold resistant *Brassica napus* and cold stress signaling

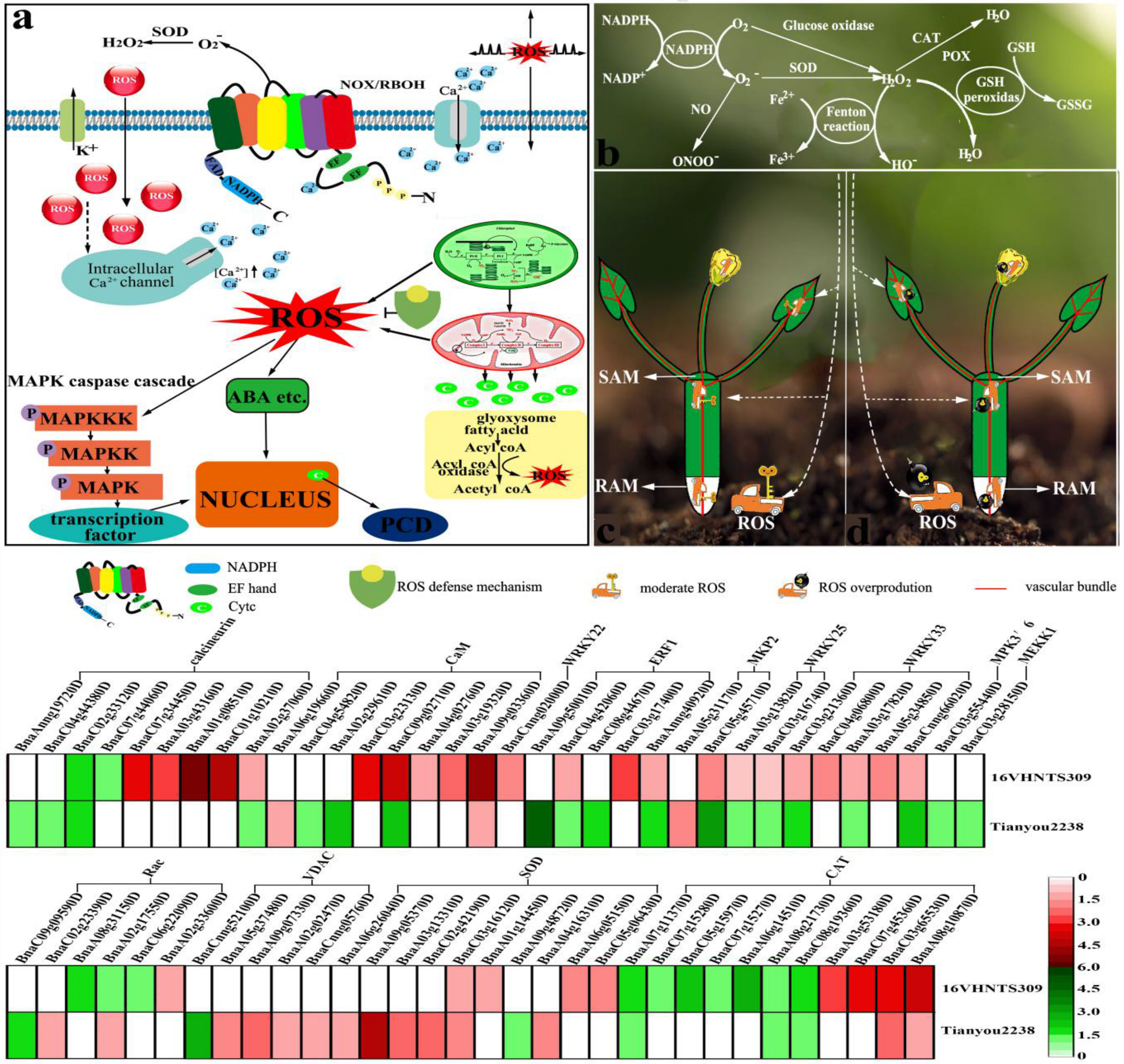


After DPI treatment of *B.napus* seeds, seed germination rate and endogenous ROS content changed

dispose	Superoxide anion	hydrogen peroxide	Germination rate(%)
CK	56.14±1.06 b	3.96±0.11 b	87.65 b
0.6%H_2O_2	68.95±1.10 a	4.74±0.10 a	94.56 a
DPI	9.92±0.42 d	0.53±0.02 d	0.00 d
0.6%H_2O_2+DPI	34.47±0.60 c	2.88±0.01 c	64.75 c

- DPI inhibited the germination rate of *B.napus* seeds and reduced the production of endogenous ROS. After 4 days of 0.6% H_2O_2+DPI treatment of *B.napus* seeds, the germination rate reached 67.5%, and the content of ROS (H_2O_2 and O_2^-) was significantly increased by 20% and 30% compared with DPI treatment.
- It was further proved that external application of H_2O_2 was opposite to DPI treatment, and that NADPH-mediated endogenous ROS played a key role in regulating the germination of *B.napus* seeds.

7. Analysis of pathways related to ROS generation, clearance and signaling during cold response of strong winter cold resistant *Brassica napus*.



- Transcriptome data, physiological and biochemical data, and cell data showed that 16HNTS309 ROS, which has strong cold resistance, has strong clearance ability.
- Strong cold-resistant material 16HNTS309: Appropriate amount of ROS interacts with related Ca^{2+}, MAPK, transcription factor (WAKY), ABA, H_2S and other signaling pathways to form a complex signal regulation mechanism to adapt to cold stress environment.
- Weak cold-resistant material Tianyou 2238: Excess ROS, upregulation of mitochondrial pore protein (VDAC) expression, promote the release of apoptotic factors such as Cyto c, which in turn induces PCD occurrence, such as obvious cell structure destruction.

Acknowledgements

We tha … cience, the
China Agri … oundation of
China, Scie … old and Arid
Agricultur …
Dr. Li N …

			
Name	W…	Yuanyuan Pu	Gang Yan
Major work	Prof… Tea…	Dr, Molecular Mechanisms of Cold Resistance	Dr, Studies on the mechanism of self-incompatibility in winter rapeseed

Thanks for your listening!