Plant Archives Vol. 25, Supplement 2, 2025 pp.1554-1558 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.supplement-2.195

UNLEASHING THE POTENTIAL OF HYDROGEN PEROXIDE (H_2O_2) TO ENHANCE GROWTH, SEED QUALITY, AND DISEASE RESISTANCE IN ONION (*ALLIUM CEPA* L.)

Arushi Padiyal[1&2*], Narender K. Bharat[2], Ravi Pratap[1] and Mohit Kamboj[1*]
[1]Department of Agriculture, Maharishi Markandeshwar (Deemed to be University), Mullana, Ambala, Haryana, India
[2]Department of Seed Science & Technology, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India
*Corresponding authors E-mail: aru.padiyal@gmail.com; kambojmohit21@gmail.com
(Date of Receiving : 24-05-2025; Date of Acceptance : 25-07-2025)

ABSTRACT

Onion (*Allium cepa* L.) is a vegetable crop which frequently challenged by diverse biotic and abiotic stresses. To investigates the efficacy of hydrogen peroxide (H_2O_2) formulations, as a sustainable alternative to chemical fungicides, for improving seed quality, plant growth, and disease resistance in onion. A study was conducted over two consecutive years (2021-2023), covering laboratory conditions at Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Himachal Pradesh. Onion seeds cv. 'Palam Lohit' were primed with different H_2O_2 concentrations and durations, and assessed using standard seed quality tests, disease incidence evaluations in randomized experimental designs. H_2O_2 treatments significantly improved germination, vigor, seedling growth, and yield, while reducing the incidence of major onion pathogens such as *Alternaria*, *Fusarium*, *Penicillium* and *Aspergillus* spp. These results highlight H_2O_2 as a promising eco-friendly solution for onion productivity and health, reducing reliance on conventional pesticides and mitigating related environmental and health risks.
Keywords: Onion, Seed Priming, Hydrogen Peroxide, Eco-friendly Solution, Conventional Pesticides.

Introduction

Allium cepa is a globally significant vegetable crop which belongs to Amaryllidaceae family. It possesses immense functional properties including anti-inflammatory, antioxidants, anticarcinogenic, immunomodulatory, anti-obesity, antimicrobial and cardiovascular protective due to presence of high concentrations flavonoids, organo-sulphur compounds, phenolics, saponins and other bioactive compounds (Lee *et al.,* 2025). However, onion production is threatened by various diseases, including purple blotch, Stemphylium blight, downy mildew, Fusarium basal rot, bacterial soft rot, and viral infections, along with abiotic stresses such as poor seed quality, water stress, and nutrient deficiencies (Hasan, 2020). Traditional reliance on chemical fungicides has resulted in environmental pollution, health hazards, and resistance development among pathogens, necessitating sustainable crop management alternatives. In this search, H_2O_2 is emerging as a potent plant growth regulator signaling molecule and disease resistance inducer which plays a pivotal role in enhancing abiotic stress tolerance. It modulates various physiological processes including photosynthesis and stress-responsive pathways such as reactive oxygen species (ROS) and methylglyoxal detoxification (Gondim *et al*., 2013; Hossain and Fujita, 2013; Wang *et al*., 2014) and its lower concentrations during seed priming improve seed invigoration and overall performance by enhancing superoxide dismutase (SOD) activity and nutrient content in roots and shoots (Ahmad *et al*., 2015). Its role in other crops is well documented includes modulation of antioxidant enzymes, defense response signaling, pathogen inhibition, and enhancement of seed vigor and stress tolerance. Despite the recognized importance of H_2O_2 in plant physiology, its specific role in regulating growth, seed quality parameters, and disease resistance in onion remains largely unexplored, with very limited research

addressing these aspects. This evident scarcity of comprehensive studies highlights a significant knowledge gap, warranting the present investigation to systematically elucidate the influence of H_2O_2 on onion crop performance. Compared to synthetic chemicals, H_2O_2 is both eco-friendly and rapidly degradable, offering multiple benefits in horticultural production. In light of the aforementioned facts, the present study aimed to investigate the role of H_2O_2 in promoting growth, seed quality, and disease resistance in onion crop as a sustainable strategy.

Materials and Methods

Location and Experimental Details

The experiments were conducted under laboratory conditions (2021-2023) at the Department of Seed Science and Technology, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (30.51°N, 77.09°E; Elevation 1,183m), within the sub-temperate, semi-humid mid-hills of Himachal Pradesh. The soil prior to trials had suitable pH (7.04-7.74), high organic carbon, and adequate macro-nutrients for crop production.

Procurement of Seeds and Chemicals

Onion cv. 'Palam Lohit' seeds were sourced from the Department of Seed Science and Technology, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, HP. Analytical grade H_2O_2 solutions and standard laboratory reagents *e.g.,* sodium hypochlorite, ethanol were used. Seeds were surface-sterilized with 1% NaOCl, rinsed, then subjected to H_2O_2 priming as detailed below.

Preparation of H_2O_2 Solution for priming

H_2O_2 solutions were prepared at concentrations ranging from 40-120 ppm for seed priming by mixing 40, 80 and 120 mg of H_2O_2 in small amount of distilled water and stirred until dissolved completely. The final volume of the solution was made up to 1000 ml by diluting it with more distilled water (Kazemi, 2014). Seeds were soaked in these H_2O_2 solutions (5x seed weight) at room temperature for 6, 12, and 24 h and shade-dried before sowing.

Laboratory Trials

A Completely Randomised Design (CRD) with 4 replications was used to determine optimal H_2O_2 priming doses and durations. Standard ISTA protocols were employed, assessing: germination %, germination speed and seedling length/weight, seed vigor indices I & II and seed-associated microflora.

Three laboratory methods were employed to study onion seed quality and microflora association. The rolled paper towel method (Anonymous, 1996) was used to evaluate seed germination and vigor, in which 100 treated seeds were placed between moist germination papers, rolled, and incubated at 25° C for 12 days, and then evaluated. The blotter paper test or Standard Petri Plate method (ISTA, 2005) was followed for microflora association in which 25 seeds were placed on one or more layers of moist blotter papers in transparent Petri plates, and these plates with seeds were incubated at 25 °C temperatures for 12 days, and examined microscopically for pathogen presence. The agar plate method involved placing seeds on sterilized potato dextrose agar in Petri plates, incubating them at 28 ± 2°C, and identifying fungal colonies microscopically based on growth characteristics.

The parameters observed under laboratory conditions included germination (%) which was tested using the paper towel method and counts were taken on the 6^{th} and 12^{th} days. Germination per cent was calculated based on the number of normal seedlings emerged from the total sown seeds. Speed of germination was calculated by daily recording the number of seedlings emerged and calculated as:

Speed of gemination = n1/d1 + n2/d2 + n3/d3 +……+ n12/d12

(where, n = number of germinated seeds, d = number of days)

Seedling length was measured from the shoot tip to the root tip of 10 randomly selected seedlings after 12 days, with a scale and mean values were expressed in centimetres (cm) and were oven dried at 60° C for 48 h, and the mean seedling dry weight was recorded in milli gram (mg). Seed vigour index-I and II were calculated as per the formulas given by Abdul-Baki and Anderson (1973).

The pathogens associated with seeds were observed using the Standard Petri Plate method and the number of infected seeds were recorded daily and per cent incidence was calculated.

Statistical Analysis

Data were analyzed using ANOVA via R software (v4.2.1), with post-hoc significance considered at $p \leq 0.05$. Results were visually summarized using appropriate charts and graphs.

Results

Effect of seed priming on seed quality parameters

Seed priming with H_2O_2 led to a significant enhancement in seed quality parameters, as presented in Table 1. Germination percentage increased considerably from 71.00% in untreated seeds to

83.00% in seeds primed with 40 ppm H_2O_2 for 12 h. Similarly, the speed of germination improved from 17.34 in untreated seeds to 22.89 in seeds subjected to 40 ppm H_2O_2 for 12 h. Comparable, statistically significant increases in both germination percentage and speed of germination were also observed in seeds primed with 40 ppm H_2O_2 for 6 h and with 80 ppm H_2O_2 for 12 h. Further improvements were noted in additional seedling parameters. Seedling length (12.35 cm), seedling dry weight (2.63 mg), seedling vigor index I (SVI–I, 1024.46) and seedling vigor index II (SVI–II, 217.68) were highest in seeds primed with 40 ppm H_2O_2 for 12 h and lowest in untreated seeds. The treatments 80 ppm H_2O_2 for 12 h and 40 ppm H_2O_2 for 6 h also exhibited strong positive effects, closely following the best treatment.

Analysis of variance indicated that Factor A (H_2O_2 concentration) exerted a more pronounced effect on most seed quality parameters, particularly on germination percentage (KA = 6.92) and seedling vigor indices. This suggests that the concentration of H_2O_2 plays a crucial role in seed metabolic activation. In contrast, Factor B (priming duration) had a relatively smaller, though still significant, influence most notably on seedling length and speed of germination indicating that appropriate priming duration enhances physiological responses, whereas excessive exposure time may negatively affect seed performance.

Effect of seed priming with H_2O_2 on pathogens associated

The lowest association of pathogens (Table 2) including *Alternaria*, *Fusarium*, *Penicillium* and *Aspergillus* spp. were recorded in the seeds primed with 40 ppm H_2O_2 for 12 h having respective incidences of 0.75, 0.75, 1.50 and 1.75%. The statistically similar reduction in incidence of *Alternaria* was recorded in the seeds primed with 80 ppm H_2O_2 for 12 h. However, 40 ppm H_2O_2 for 12 h was the only best performing treatment for *Fusarium* incidence. On treating seeds with 40 ppm H_2O_2 for 6 as well as 24 h also significantly reduced the association of *Penicillium* spp. Comparatively more treatments *viz.,* 40 and 80 ppm H_2O_2 for 6 and 24 h were effective in reducing incidence of *Aspergillus* spp. Besides, the ranking analysis of the data revealed that Factor A (H_2O_2 concentration) had a greater impact in reducing seed-borne microflora, especially *Alternaria*, *Fusarium*, and *Penicillium spp.*, as indicated by lower KA values (KA = 1.75–2.5). This shows that increasing concentration was more effective in suppressing fungal contamination, while Factor B (priming duration) showed lesser but consistent effects, with the lowest KB values (KB = 0.83–1.25) for all pathogens, suggesting that shorter durations of priming were more effective in minimizing fungal growth, while longer durations may begin to lose effectiveness or cause rebound contamination.

Discussion

Hydrogen peroxide (H_2O_2) treatment significantly improved seed quality under present study, because of its well documented role as a signaling molecule in seeds to activate transduction pathways for removing hinderance during germination including pericarp inhibitor oxidation, seed coat scarification, or both, resulting in rapid and more uniform germination (Wojtyla *et al.,* 2016). Besides, H_2O_2 is known to elevate the antioxidant enzyme activity like catalase and superoxide dismutase which scavenge the reactive oxygen species (ROS) (Hossain *et al.,* 2018) produced during metabolic processes occurring in germinating seed which leads to reduction in oxidative damage caused to membrane lipids and proteins, thus actively repairing the membrane structures disrupted during seed desiccation which in turn reduce the leakage of electrolytes, maintaining the membrane integrity and stability for efficient imbibition of water and nutrient mobilization on being primed with H_2O_2 (Zhang *et al.,* 2021) . These results are well validated by study of Ahmad *et al*. 2015 who demonstrated that lower H_2O_2 concentrations (20 mg L^{-1} or 40 mg L^{-1}) during seed priming enhanced maize germination and performance. Moreover, the best results observed on priming with 40 ppm for 12 h could be attributed to the sufficient time provided to the seed in H_2O_2 for enzyme and antioxidants activation, dormancy break and membrane repair, which could have been less efficient in shorter durations causing incomplete activation and oxidative damage during longer durations or higher concentrations. These results in line with study of Kuˇcerová *et al*. (2021) who reported that seeds when primed higher H_2O_2 levels negatively impacted leaf size, shape and colour in lettuce.

Hydrogen peroxide (H_2O_2) acts as oxidizing agent for fungal spores and mycelia, hindering their establishment on or in the seed and also causes disruption of their cell walls (Wojtyla *et al.,* 2016). It also triggers innate defense mechanism of the seed via activation of antioxidant enzymes, upregulation of pathogenesis-related proteins and other defense responses through the redox signaling pathway (Gierczik *et al*., 2020). Our results can be backed by several similar studies on different crops like pearl millet (Geetha and Shetty, 2002), onion (Słupinska, 2012), and carrot (Szopińska *et al*., 2017), where H_2O_2 application effectively suppressed fungal development in seeds. The best results observed in seeds primed

with 40 ppm H_2O_2 might be because of optimal oxidative dose that penetrates deep enough to suppress pathogens like *Fusarium* without harming seed tissues, allowing activation of antioxidant defense and membrane repair. In contrast, higher concentrations (80 ppm) may control tougher fungi like *Alternaria* but risk seed stress, while *Penicillium* and *Aspergillus*, being surface-borne and more ROS-sensitive, respond well across multiple durations and concentrations.

Conclusion

Hydrogen peroxide seed priming and foliar sprays offer reliable, effective, and sustainable enhancements for onion crop establishment, seedling vigor, disease resistance, yield, and seed quality. This strategy presents a robust alternative to chemical pesticides and fungicides, minimizes risks to human/livestock health and the environment, and helps meet the growing demand for eco-friendly horticultural management. Further long-term studies on cost-effectiveness, compatibility with integrated crop management systems, and field scalability are recommended.

Table 1 : Effect of H_2O_2 Priming Duration and Concentration on Onion Seed Quality and Vigor

Treatments	Concentration (ppm)	Priming duration (h)	Seed quality parameters					
			Germination (%)**	Speed of germination	Seedling length (cm)	Seedling dry wt. (mg)	SVI – I (Length)	SVI – II (Mass)
H_2O_2	40	6	81.00±1.53^{ab}	22.10±0.78^{ab}	12.04±0.14^{ab}	2.51±0.10^{bc}	974.95±5.27^{b}	203.06±8.42^{b}
		12	83.00±1.27^{a}	22.89±0.45^{a}	12.35±0.30^{a}	2.63±0.03^{a}	1024.46±27.70^{a}	217.68±0.98^{a}
		24	78.25±0.14^{bcd}	21.22±0.52^{bc}	11.7±0.18^{bc}	2.46±0.08^{bcd}	914.97±8.82^{c}	192.61±3.13^{c}
	80	6	78.00±0.70^{cd}	21.31±0.44^{bc}	11.57±0.25^{c}	2.44±0.10^{cd}	902.63±17.90^{c}	190.22±4.10^{cd}
		12	80.50±0.80^{abc}	21.58±0.78^{b}	12.30±0.43^{a}	2.57±0.09^{ab}	989.57±30.33^{ab}	207.12±3.36^{b}
		24	76.00±2.74^{de}	20.38±0.15^{cd}	11.26±0.12^{c}	2.39±0.03^{de}	855.46±16.19^{d}	181.91±6.89^{d}
	120	6	75.75±2.73^{de}	19.83±0.55^{de}	11.37±0.31^{c}	2.47±0.01^{bcd}	860.66±13.96^{d}	187.10±3.37^{cd}
		12	73.25±0.66^{ef}	19.56±0.25^{de}	10.63±0.11^{d}	2.30±0.03^{e}	778.52±25.96^{e}	168.50±4.56^{e}
		24	72.50±2.81^{f}	19.29±0.77^{e}	10.47±0.34^{d}	2.30±0.06^{e}	758.26±25.97^{e}	166.56±6.76^{e}
Untreated (Control)			71.00±1.60f	17.34±0.44^{f}	9.75±0.29^{e}	2.17±0.06^{f}	687.39±22.31^{f}	152.99±3.03^{f}
Ranking analysis								
		K_{a1}	80.75	22.07	12.03	2.53	971.46	204.45
		K_{a2}	78.17	21.09	11.71	2.47	915.89	193.08
		K_{a3}	73.83	19.56	10.82	2.36	799.15	174.05
		K_A	**6.92**	**2.51**	**1.21**	**0.18**	**172.31**	**30.4**
		K_{b1}	78.25	21.08	11.66	2.47	912.75	193.46
		K_{b2}	78.92	21.34	11.76	2.5	930.85	197.77
		K_{b3}	75.58	20.3	11.14	2.38	842.9	180.36
		K_B	**3.33**	**1.05**	**0.62**	**0.12**	**87.95**	**17.41**

Note: Factor A and B represent the effects of H_2O_2 concentration and priming duration, respectively. Ka1, Ka2, and Ka3 are the average values of each parameter for priming durations of 6 h, 12 h, and 24 h at 40 ppm H_2O_2, respectively, while Kb1, Kb2, and Kb3 represent the corresponding values for 40, 80 and 120 ppm H_2O_2. Ka and Kb indicate the mean performance across all durations for 40 ppm, 80 ppm and 12o ppm treatments, respectively. KA and KB denote the difference between the highest and lowest values of Ka and Kb for each parameter. Data are represented as mean ± standard deviation of three replicates. Mean values within a column followed by different superscript letters (a, b, c, etc.) are significantly different at $p \leq 0.05$.

Table 2 : Effect of H_2O_2 Priming Duration and Concentration on Fungal Incidence in Onion Seed

Treatments	Concentration (ppm)	Priming duration (h)	Seed microflora (%)			
			Alternaria spp. *	*Fusarium* spp. *	*Penicillium* spp. *	*Aspergillus* spp. *
H_2O_2	40	6	2.00±0.01^{g}	1.50±0.03^{h}	1.75±0.02^{i}	2.75±0.10^{g}
		12	0.75±0.03^{j}	0.75±0.02^{i}	1.50±0.02^{j}	1.75±0.07^{i}
		24	1.75±0.06^{h}	2.00±0.08^{f}	2.50±0.07^{h}	3.00±0.12^{f}
	80	6	2.25±0.08^{f}	2.75±0.10^{e}	3.25±0.13^{e}	2.50±0.11^{h}
		12	1.50±0.01^{i}	1.75±0.05^{g}	2.75±0.12^{g}	2.50±0.03^{h}
		24	2.75±0.11^{e}	3.00±0.05^{d}	4.00±0.03^{d}	3.50±0.03^{e}
	120	6	3.00±0.11^{d}	3.75±0.11^{c}	3.00±0.04^{f}	4.00±0.02^{d}
		12	3.25±0.12^{c}	3.75±0.10^{c}	5.00±0.17^{c}	4.50±0.13^{c}
		24	3.50±0.04^{b}	4.00±0.02^{b}	5.25±0.23^{b}	5.00±0.17^{b}
Untreated (Control)			5.25±0.17^{a}	5.50±0.16^{a}	6.75±0.24^{a}	7.00±0.28^{a}

Ranking analysis						
		K_{a1}	1.5	1.42	1.92	2.5
		K_{a2}	2.17	2.5	3.33	2.83
		K_{a3}	3.25	3.83	4.42	4.5
		K_A	**1.75**	**2.42**	**2.5**	**2**
		K_{b1}	2.42	2.67	2.67	3.08
		K_{b2}	1.83	2.08	3.08	2.92
		K_{b3}	2.67	3	3.92	3.83
		K_B	**0.83**	**0.92**	**1.25**	**0.92**

Note: Factor A and B represent the effects of H_2O_2 concentration and priming duration, respectively. Ka1, Ka2, and Ka3 represent the average values of each seed microflora parameter for priming durations of 6 h, 12 h, and 24 h at 40 ppm H_2O_2, respectively, while Kb1, Kb2, and Kb3 represent the corresponding values for 40, 80 and 120 ppm H_2O_2. Ka and Kb denote the mean performance across all durations for 40 ppm, 80 ppm and 120 ppm treatments, respectively. KA and KB indicate the range (difference between highest and lowest Ka and Kb values, respectively) of each parameter. Data are represented as mean ± standard deviation of three replicates. Mean values within a column followed by different superscript letters (a, b, c, etc.) are significantly different at $p \leq 0.05$.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Arushi Padiyal: Conceptualization, data collection, analysis, writing of the original manuscript. N.K. Bharat: Conceptualization, planning, monitoring, editing, statistical analysis. All other authors: Writing and editing the final version of the manuscript.

References

Ahmad, I., Hussain, S., Hussain, S., Rehman, A., Ali, A., Hussain, S., Basra, S. and Rehmani, M. I. A. (2015) Priming with ascorbic acid, salicylic acid and hydrogen peroxide improves seedling growth of spring maize at suboptimal temperature. *Journal of Environmental Agriculture and Sciences,* **3**: 14–22.

Anonymous. (1996) International rules for seed testing. Seed Science and Technology 24: 1–335.

Geetha, H. M. and Shetty, H. S. (2002) Induction of resistance in pearl millet against downy mildew disease caused by Sclerospora graminicola using benzothiadiazole, calcium chloride and hydrogen peroxide – a comparative evaluation. *Crop Protection,* **21**: 601–610.

Gierczik, K., Vukušić, T., Kovács, L., Székely, A., Szalai, G., Milošević, S., Kocsy, G., Kutasi, K. and Galiba, G. (2020) Plasma-activated water to improve the stress tolerance of barley. *Plasma Processes and Polymers,* **17**: 1900123.

Gondim, F. A., Miranda, R. S., Gomes-Filho, E. and Prisco, J. T. (2013) Enhanced salt tolerance in maize plants induced by H_2O_2 leaf spraying is associated with improved gas exchange rather than with non-enzymatic antioxidant system. *Theoretical and Experimental Plant Physiology,* **25**: 251–260.

Hasan, M. (2020) Management of purple blotch complex of onion (*Allium cepa*) for seed production. Doctoral dissertation, Department of Plant Pathology.

Hossain, M. A. and Fujita, M. (2013) Hydrogen peroxide priming stimulates drought tolerance in mustard (*Brassica juncea* L.). *Plant Gene Trait,* **4**: 109–123.

Hossain, M. A., Bhattacharjee, S., Armin, S. M., Qian, P., Xin, W., Li, H. Y., Burritt, D. J., Fujita, M. and Tran, L. S. P. (2015) Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: Insights from ROS detoxification and scavenging. *Frontiers in Plant Science,* **6**: 420.

International Seed Testing Association. (2005) International rules for seed testing. ISTA, Zurich, Switzerland.

Kazemi, M. (2014) Effect of foliar application with salicylic acid and methyl jasmonate on growth, flowering, yield and fruit quality of tomato. *Bulletin of Environment, Pharmacology and Life Sciences,* **3**: 154–158.

Kučerová, K., Henselová, M., Slováková, L., Bačovčinová, M. and Hensel, K. (2021) Effect of plasma activated water, hydrogen peroxide, and nitrates on lettuce growth and its physiological parameters. *Applied Sciences,* **11**: 1–13. https://doi.org/10.3390/app11051985

Lee, H. J., Lee, I. Y., Park, J. H. and Joo, N. (2025) Comparison of phytochemicals and antioxidant activities of onion (*Allium cepa* L.) bulbs, onion leaves, and green onion (*Allium fistulosum* L.) leaves. *International Journal of Food Properties,* **28**(1): 2507121.

Słupinska, E. (2012). Effects of hydrogen peroxide and organic acids on germination, vigour and health of onion (*Allium cepa* L.) seeds. MSc thesis, Poznań University of Life Sciences, Poland (in Polish with English summary).

Szopińska, D., Jarosz, M. and Sławińska, B. (2017). The effect of hydrogen peroxide on seed quality and emergence of carrot (*Daucus carota* L.). *Acta Scientiarum Polonorum Hortorum Cultus,* **16**: 21–33.

Wang, Y., Zhang, J., Li, J. L. and Ma, X.R. (2014). Exogenous hydrogen peroxide enhanced the thermotolerance of *Festuca arundinacea* and *Lolium perenne* by increasing the antioxidative capacity. *Acta Physiologiae Plantarum,* **36**: 2915–2924.

Wojtyla, Ł., Lechowska, K., Kubala, S. and Garnczarska, M. (2016) Different modes of hydrogen peroxide action during seed germination. *Frontiers in Plant Science,* **7**: 66.

Zhang, K., Zhang, Y., Sun, J., Meng, J. and Tao, J. (2021). Deterioration of orthodox seeds during ageing: Influencing factors, physiological alterations and the role of reactive oxygen species. *Plant Physiology and Biochemistry,* **158**: 475–485.