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抗坏血酸对种子贮藏潜力的保持

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摘　要：用一种选定的化学物质对一种黑克兰种子活力的延长进行了研究。在加速老化条件下，黑克种子快速丧失活力。在加速老化处理（100% RH和32±2℃）之前，用抗坏血酸预处理黑葡萄（Vigna mungo L.）种子6小时（3+3），持续不同的时间（0至30天），可减缓老化引起的快速发芽损失。该化学物质还显著抑制了种子种子在强制老化期间蛋白质、不溶性碳水化合物水平的降低以及过氧化氢酶活性的降低，在化学预处理的种子中显著改善。相反，种子预处理剂可以减轻老化诱导的淀粉酶活性的刺激。尽管存在不利的贮藏条件，但处理诱导了更高的蛋白质和过氧化氢酶活性，这证明了种子潜力在预处理中要好得多。因此，结果表明，抗坏血酸预处理的种子保持了黑克兰物种更高的种子活力。在这项研究中，实验化学物质对种子的贮藏增强作用是明显的。

关键词：黑革兰；抗坏血酸；过氧化氢酶；种子电位；加速老化

Retention of Seed Storage Potential Using Ascorbic Acid

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Abstract: An investigation was carried out on prolongation of seed vigour of a black gram species by using a selected chemical. Black gram seeds lost viability at a rapid pace under accelerated ageing condition. Pretreatment of black gram (Vigna mungo L.) seeds with ascorbic acid for 6 hours (3+3) before accelerated ageing treatment (100% RH and 32±2℃) for different durations (0 to 30 days) slowed down the ageing-induced rapid loss of germination. The chemical also significantly arrested the reduction of protein, insoluble carbohydrate levels as well as activity of catalase enzyme of seed kernels during forced ageing period was ameliorated to a significant extent in the chemical-pretreated seed. Conversely, ageing-induced stimulation of the activity of amylase enzyme was alleviated by the seed pretreating agent. Seed potential was found to be much better in the pretreatments as evidenced from the treatment-induced higher protein and activity of catalase enzyme in spite of adverse storage situation. Results, therefore, pointed out that the ascorbic acid pretreated seeds retained higher seed vigour of black gram species. The promising effects of the experimental chemical on storage potentiation of the seed is apparent in this investigation.

Keywords: Black gram; Ascorbic acid; Catalase; Seed potential; Accelerated ageing

1. 简介

自农业文明衰落以来，保持种子活力一直是人类极为关注的问题。优质种子是农业成功的关键。农业要求每一粒种子都要容易发芽，并产生一株活力四射的幼苗，确保高产[1]。在热带和亚热带国家，种子的储存是一个严重的问题，在这些国家，高温和高相对湿度极大地加速了种子老化现象，从而导致种子退化和丧失活力。印度许多邦的种子活力保持问题更为严重，因为其半干旱气候，一年中大部分时间相对湿度较高，非常有利于微生物，尤其是真菌的生长[2，3]。这两种环境因素严重损害了种子和幼苗的健康，并导致种子发芽率和幼苗性能快速下降[4，5]。因此，印度种植者经常被迫在农业中使用低活力种子。为了解决这个问题，目前正在采取策略来提高种子的储存潜力，以延长其寿命[6-8]。

考虑到种子储存的问题，在这项研究中，我们试图延长黑克物种的储存寿命。本研究的实验在加速老化条件下进行，以获得或多或少均匀和快速的结果。事实上，由高温和高相对湿度（RH）强加的加速老化处理为研究

种子在极短时间内的劣化过程提供了有力的工具[9-11]。

因此，这项工作的主要目的是通过分析种子的发芽行为和代谢状态，探索试验化学品对提高黑克兰种子活力的功效。

2. 材料和方法

表面灭菌（0.1%HgCl2，90秒）后，将黑克（Vigna mungo L.）种子样品分别预浸在抗坏血酸水溶液（100μg ml–1）或蒸馏水中3小时（h），然后干燥至种子的原始干重。重复两次，使水溶液中存在的化学品最大限度地渗透。将预处理过的种子批次（每次处理200g）装入单独的布袋中，并储存在预先施加100%相对湿度（RH）的干燥器中。该实验装置在32±2℃下保持30天，使种子经历强制老化处理。

为了分析发芽率，将四组100粒种子，即每种处理的400粒种子转移到含有用蒸馏水润湿的滤纸的独立培养皿中。根据国际种子测试规则[12]，在浸种96小时后记录发芽数据。按照Coolbear等人描述的方法确定种子50%发芽的时间（T_{50}）[13]。

分析了籽粒中蛋白质、不溶性碳水化合物含量以及过氧化氢酶和淀粉酶活性。不溶性碳水化合物的定量按照McCready等人的方法进行[14]。根据Lowry等人的方法估计蛋白质水平[15]。按照Snell和Snell[16]的方法提取和估计过氧化氢酶。根据Khan和Faust的方法估计淀粉酶活性[17]。为了测定这些酶，将空白作为零时间对照，活性表示为（ΔOD x TV）/（txv），其中ΔOD是空白和样品的OD之差。TV是滤液的总体积，t是与底物孵育的时间（min），v是孵育所需的滤液体积[18]。

在治疗和复制水平上对数据进行统计分析，并在95%置信限下计算最小显著差异（LSD）值[19]。

3. 结果和讨论

实验结果清楚地表明，用抗坏血酸水溶液预处理种子物种显著减轻了加速老化引起的发芽损失，并缩短了T50小时（表1），减缓了不溶性碳水化合物的快速浸出（表2），减轻了蛋白质（表2）以及过氧化氢酶和淀粉酶（表3）的损失。化学诱导的所有这些有害影响的显著改善表明在不利的贮藏环境下种子增强。

膜损伤的减少可能在种子退化中起重要作用，这一观点得到了溶质泄漏以及发芽率和活力损失的研究支持[20-22]。当干燥的组织重新水合时，种子快速识别其膜的能力是成功发芽的关键因素，这在文献中有明确记载[23]。已有大量证据表明，发芽胚胎中的膜状态是恶化的重要因素[24]。因此，结果指出，尽管变质是黑克种子品种的处理和对照样品中的常见现象，但处理后的种子样品中的分解代谢过程仍然有些平缓，从而使其对不利的储存环境具有耐受性。现有报告显示，在种子老化过程中，一些重要的细胞成分（包括蛋白质、碳水化合物）发生了损失[25]。过氧化氢酶被视为一种清除剂酶，该酶的较高活性表明植物活力较高[26]。在这项研究中，化学诱导的酶活性快速丧失的阻止表明，在不利的储存条件下，化学物质加强了防御机制。

4. 结论

从本研究的结果可以得出结论，抗坏血酸水溶液可以有效地提高黑葡萄籽的贮藏潜力。因此，从这些实验结果来看，本发明种子预处理剂的诱导性能似乎很明显。

表1 用抗坏血酸水溶液（100μg ml^{-1}）预处理种子对黑克种子发芽率和T_{50}小时（50%发芽所需时间）值的影响

Seed sample	Treatments	Percentage seed germination			T_{50} of germination		
		Days after accelerated ageing					
		0	15	30	0	15	30
Black gram	Control	100	78	38	12	36	NA
	Ascorbic acid	100	80	52	12	24	84
	LSD (P = 0.05)	NC	5.58	4.38	NC	2.50	6.05

种子用化学或蒸馏水的水溶液预浸6小时，然后干燥回原始种子重量。这重复了两次。

将预处理的种子样品保持在100%相对湿度下，并在零（0）、15和30天加速老化后记录数据。

NC：未计算；NA：未达到50%发芽率。

表2 用抗坏血酸水溶液（100μg ml^{-1}）预处理种子对黑克种子蛋白质（mg/g fr.wt.）和不溶性碳水化合物（mg/g fr.wt.）水平的影响

Seed sample	Treatments	Protein			Insoluble carbohydrates		
		Days after accelerated ageing					
		0	15	30	0	15	30
Black gram	Control	61.31	40.33	19.87	23.10	18.50	10.19
	Ascorbic acid	61.09	48.12	28.99	23.16	20.19	17.07
	LSD (P = 0.05)	NS	3.67	1.49	NS	1.07	0.06

处理和数据记录如表1所示。

NC：未计算；NS：不显著。

表3 用抗坏血酸水溶液（100μg ml^{-1}）预处理种子对过氧化氢酶活性的影响（ΔODxTv/txv）和淀粉酶（ΔODxTv/txv）

Seed sample	Treatments	Catalase			Amylase		
		Days after accelerated ageing					
		0	15	30	0	15	30
Black gram	Control	40.40	26.20	16.90	37.10	50.00	67.80
	Ascorbic acid	40.00	30.90	25.00	37.00	41.20	53.40
	LSD (P = 0.05)	NS	2.20	1.15	NS	3.05	2.70

处理和数据记录如表1所示。

NS：不显著。

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