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# 基于 ATR 的玉米种子活力太赫兹特征波段筛选方法

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**摘要:** 应用太赫兹时域光谱技术结合移动窗口相关系数法筛选与种子老化密切相关的太赫兹特征波段。选取 3 个不同品种的玉米种子, 并制备人工老化 0、1、2、3、4 d 的实验样本, 采用衰减全反射 (Attenuated total reflection, ATR) 附件采集从样本分离得到的种胚和胚乳粉末的太赫兹吸光度谱 ( $0.2 \sim 80 \text{ cm}^{-1}$ ), 应用移动窗口相关系数法 (窗口宽度为 10, 阈值为 0.3) 筛选样本种胚和胚乳特征谱区。结果表明, 不同品种样本筛选得到的老化特征谱区差异显著, 说明种子老化进程受品种影响较大; 在相同的老化阶段, 不同品种样本的特征谱区也存在部分共同区间, 且主要集中在  $60 \sim 80 \text{ cm}^{-1}$ , 说明老化进程中有些成分的变化相近; 种胚和胚乳在相同老化阶段筛选的特征谱区不同, 说明两者在老化进程中生理变化存在差异。本研究表明太赫兹时域光谱结合化学计量学方法可用于快速表征并探测玉米种子老化的动态变化过程。

**关键词:** 种子活力; 太赫兹时域光谱; 特征波段; 衰减全反射; 移动窗口相关系数法

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## Screening Method of Characteristic THz Region to Corn Seed Vigor Based on ATR

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**Abstract:** Characteristic Terahertz (THz) region closely related to corn seed aging were screened by using THz time domain spectroscopy technique and moving window correlation coefficient method. There were three different varieties of corn seeds selected and processed by artificial aging for 0 d, 1 d, 2 d, 3 d and 4 d. The embryos and endosperms of corn seeds samples were isolated and pulverized. THz absorbance spectra ( $0.2 \sim 80 \text{ cm}^{-1}$ ) of the powder samples were collected by THz time domain system and attenuated total reflection (ATR) accessory. The moving window correlation coefficient method was applied to screen the characteristic spectral regions which were sensitive to seed aging. After comparing the parameters of the moving window correlation coefficient, the window width was determined to 10 and threshold value was 0.3. According to the result, the selected characteristic spectral regions showed significant differences among different varieties of samples, which indicated that the seed aging process was greatly affected by the variety. However, there were minor common regions in the same aging stage which were mainly concentrated in  $60 \sim 80 \text{ cm}^{-1}$ . It was showed that the similar components were changed at the same time. Due to the selected characteristic spectra of endosperm and embryo at the same aging stage were different, it was showed that the endosperm and embryo had different physiological changes during seed aging process. The experiment result indicated that THz time domain spectroscopy combined with chemometric method can be used to characterize and detect the dynamic changes of corn seed aging rapidly and macroscopically.

**Key words:** seed vigor; Terahertz time domain spectroscopy; characteristic region; attenuated total reflection; moving window correlation coefficient method

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## 0 引言

玉米是我国第一大粮食作物。高活力种子具有显著的生长优势<sup>[1-2]</sup>,玉米种子活力直接影响我国玉米种子品质安全、玉米播种方式以及玉米产量等。因此,开展玉米种子活力研究具有重要意义。

种子活力变化与老化裂变过程密切相关,随着老化程度的加剧,种子活力开始下降且不可逆转。李春雷等<sup>[3]</sup>测定了人工加速老化后郑单958和先玉335两个品种的玉米种子活力指标、种子内物质含量及生理指标,结果表明,随着老化程度的加剧,种子发芽率、发芽指数、活力指数逐渐降低,过氧化物酶、过氧化氢酶、脱氢酶的活力逐渐降低,电导率、丙二醛含量逐渐升高,粗脂肪、可溶性蛋白含量先升高、后降低,有机酸含量总体呈升高的趋势,脯氨酸含量呈现上升的趋势。张海燕<sup>[4]</sup>研究了人工老化对科糯2000和莱农糯6号玉米种子发芽指标和生理特性的影响,结果表明,随老化时间的延长,种子发芽势、发芽率、发芽指数、活力指数和脱氢酶活性逐渐降低,丙二醛含量和相对电导率逐渐升高。

上述研究均采用化学分析方法精确测定了玉米种子老化过程中某个阶段、某些理化指标的变化,并取得了一些共性的结论。但是,种子老化是极为复杂的综合、连续、动态变化过程,传统实验室分析方法大都存在单指标测定、耗时、操作繁杂、消耗化学试剂等弊端,无法实时、便捷地反映种子老化及活力综合变化过程。太赫兹(Terahertz, THz)时域光谱技术<sup>[5-10]</sup>具有低能级、穿透性和独特的波谱性,可以快速、安全、宏观地表征种子生物样本信息,因此迅速成为近年来种子品质检测领域的研究热点,如用于作物种子转基因识别<sup>[11-14]</sup>、谷物储存品质鉴别<sup>[15-18]</sup>等。本文提出采用太赫兹时域光谱技术结合化学计量学方法,筛选玉米种子老化不同阶段的太赫兹特征谱区,以期通过老化阶段特征谱区探索种子活力的动态变化过程。

## 1 材料与方法

### 1.1 实验材料

为统计不同品种的玉米种子在老化过程中是否有共性的变化规律,选取中地77、中地168、强硕68共计3个品种的玉米种子进行实验。采用种子老化箱在高温(40~50℃)、高湿(相对湿度100%)条件下对样本进行0、1、2、3、4 d人工加速老化处理,如图1所示。按照国家标准(GB/T 3543.4—1995)采用种子发芽箱进行发芽实验,如图2所示。

共计15组样本,每组进行4次重复发芽实验,



图1 人工老化实验

Fig. 1 Artificial aging experiment



图2 玉米种子发芽实验

Fig. 2 Corn seed germination experiment

取平均值作为最终发芽率,如表1所示。

表1 种子发芽率统计信息

Tab. 1 Corn seed germination rate %

玉米品种	老化时间/d				
	0	1	2	3	4
中地77	88.00	85.50	34.50	21.50	3.00
中地168	94.50	70.00	46.00	10.50	0
强硕68	89.00	84.00	53.00	10.50	1.50

由于玉米种胚及胚乳化学成分和含量存在明显差异,为观察玉米种子不同组织在老化过程中的活动变化,本实验对老化后的种胚及胚乳进行分离,并研磨成粉,制为样本备用。

### 1.2 ATR-THz 光谱采集

实验采用TeraPulse 4000型太赫兹时域光谱系统及衰减全反射(ATR)<sup>[19-22]</sup>附件采集种胚和胚乳粉末样本太赫兹吸光度谱。ATR模块入射角为35°,光谱范围设定为0.2~80 cm<sup>-1</sup>,光谱分辨率为0.94 cm<sup>-1</sup>,采样点数为338,单个样本扫描900次取平均光谱。光谱采集过程中实验环境温度保持在22℃左右,同时采用空压机吹扫系统以减少环境水分的干扰。

以中地77玉米种子样本为例采集得到不同老化阶段种胚、胚乳的ATR吸收光谱如图3所示。从图3中可以直观观测到光谱曲线轮廓相近,但是不

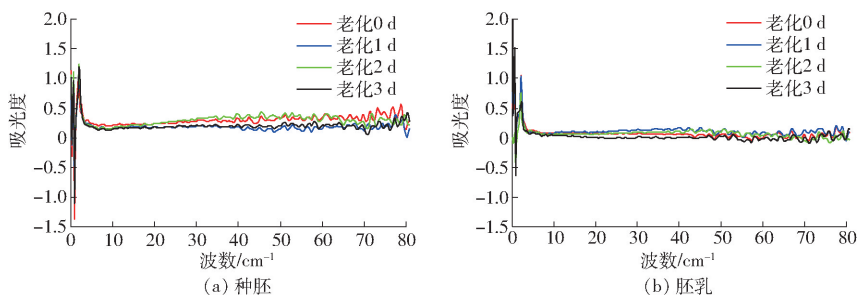


图3 中地77玉米种子不同老化阶段ATR光谱图

Fig. 3 ATR spectra of Zhongdi 77 corn seeds at different aging stages

同老化阶段的曲线又存在细微差异。

### 1.3 移动窗口相关系数法

移动窗口相关系数法<sup>[22-25]</sup>是一种用于计算光谱间相似度的方法。对于要进行相似度比较的2条光谱,在每一个移动子窗口计算出相关系数,把相关系数与对应窗口的起始位置作图,可得到移动相关系数图。若2条光谱在某一区间存在差异,则该区间的移动相关系数将明显下降;反之若完全相似,则整个光谱范围的移动相关系数都为1。基于时间序列的相关系数图可以跟踪反映光谱信息随时间微扰的变动。本文重点探索应用移动窗口相关系数法研究老化0~1d、1~2d、2~3d过程中光谱数据的变化,通过基于时间序列的相关系数图筛选与老化相关的敏感谱区,统计谱图变化规律即可以初步探索种子老化过程中活力动态变化。

## 2 结果分析

采用移动窗口相关系数法计算相邻老化阶段(0~1d、1~2d、2~3d)相关系数。若移动窗口相关系数图中相关系数偏低,则表明该相关系数对应的THz谱区窗口受老化时间扰动影响较大,筛选得到的光谱区间则是反映种子老化过程动态变化的特征谱区。

### 2.1 基于时间序列的玉米种胚相关系数谱图解析

窗口宽度是移动窗口相关系数法中的重要参数。窗口宽度过小则相关系数个数过多,可能出现多个由光谱噪声干扰导致的“伪”特征波段;窗口过大则导致滑动过快,易丢失特征波段信息。经过实

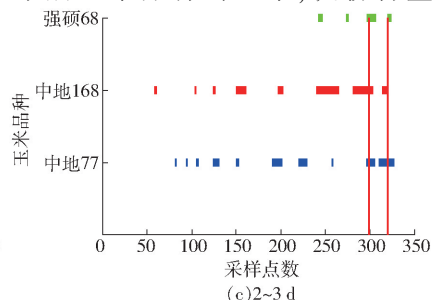
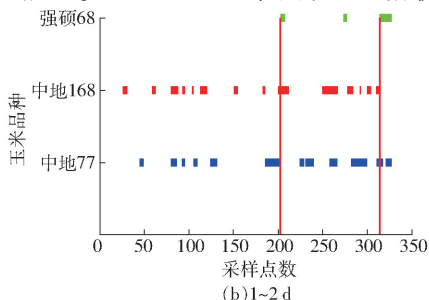
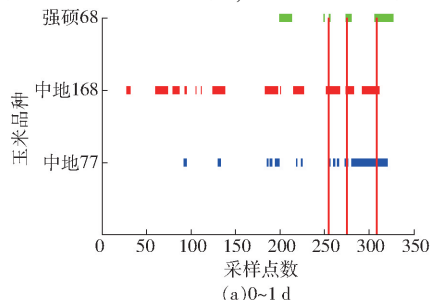


图5 3类玉米种子不同老化阶段种胚特征波段示意图

Fig. 5 Characteristic bands diagrams of three kinds of corn seeds embryos at different aging stages

验比较,设定窗口宽度为10。以中地77玉米种子为例,人工老化0~1d的种胚ATR吸收谱的移动窗口相关系数如图4所示。实验设定相关系数阈值为0.3,则相关系数小于0.3对应的谱区窗口即为受老化时间扰动较大的特征光谱区间。

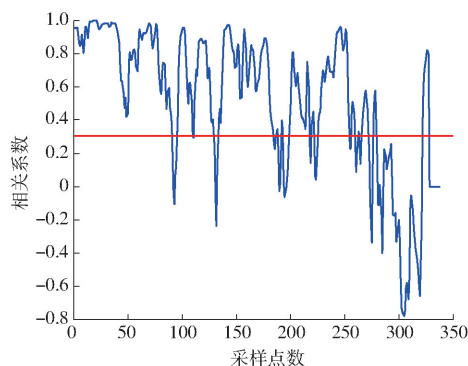


图4 中地77玉米种子种胚老化0~1d移动窗口相关系数

Fig. 4 Moving window correlation coefficient diagram of Zhongdi 77 corn seeds embryo between aging 0 d and 1 d

将所有符合上述条件的特征波段用颜色进行标识,就可以得到与老化过程密切相关的特征波段在整个光谱区间的分布示意图。图5为3个品种玉米种子样本种胚在0~1d、1~2d、2~3d老化过程中的特征波段分布。

从不同品种相同老化阶段特征波段分布分析可得,不同品种的玉米特征波段分布显著不同,如强硕68与中地77、中地168的特征波段分布迥然不同,这可能由于玉米品种不同导致的。本实验中强硕68相较于后2个品种的玉米,淀粉含量

较高。

从图5中也可以观察到不同品种的玉米在同一老化阶段存在部分共同特征谱区,如图中红色竖线所标注区域:0~1 d老化共同谱区为 $61.1 \sim 63.5 \text{ cm}^{-1}$ (采样点数为255~256)、 $65.7 \sim 68.3 \text{ cm}^{-1}$ (274~276)、 $73.4 \sim 76.9 \text{ cm}^{-1}$ (306~312);1~2 d老化共同谱区为 $48.7 \sim 50.8 \text{ cm}^{-1}$ (203)、 $75.3 \sim 77.5 \text{ cm}^{-1}$ (314);2~3 d老化共同谱区为 $71.2 \sim 74.8 \text{ cm}^{-1}$ (297~303)、 $76.7 \sim 78.9 \text{ cm}^{-1}$ (320)。不同品种的玉米在3个不同老化阶段均存在共同谱区,主要集中在 $60 \sim 80 \text{ cm}^{-1}$ 范围,实验表明尽管样本品种不同,但是在相同的老化阶段均存在某些相近的官能团或物质成分产生了共性变化。但是共同谱区所对应的种子老化或是活力变化机理还有待解析。

## 2.2 基于时间序列的玉米胚乳相关系数谱图解析

胚乳特征谱段筛选和种胚分析类似,设置窗口

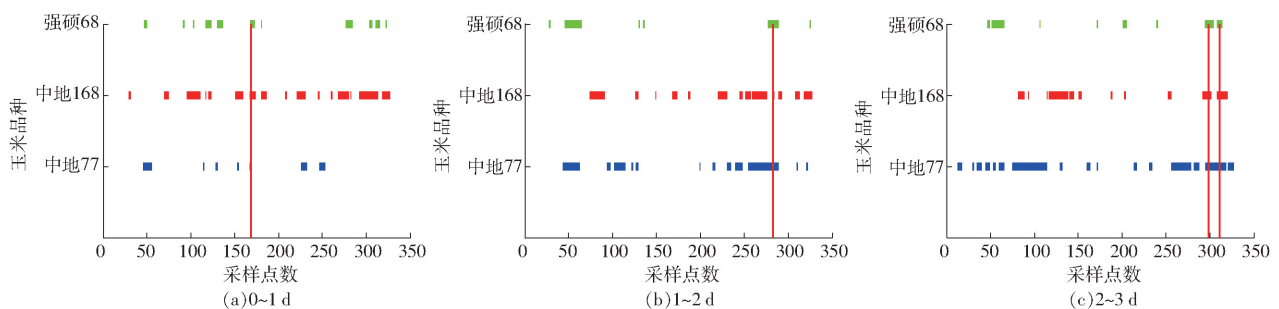


图7 3类玉米种子老化阶段胚乳特征波段示意图

Fig. 7 Characteristic bands diagrams of three kinds of corn seeds endosperm at different aging stages

与上述玉米种胚分析结果类似,不同品种的玉米筛选得到的胚乳特征波段存在明显差异,但是不同品种的玉米在同一个老化阶段也存在共同的特征谱区,如图7中红色竖线所标注区域:0~1 d老化共同谱区为 $40.3 \sim 42.9 \text{ cm}^{-1}$ (168~170);1~2 d老化共同谱区为 $67.6 \sim 70.3 \text{ cm}^{-1}$ (282~284);2~3 d老化共同谱区为 $70.7 \sim 74.6 \text{ cm}^{-1}$ (295~302)、 $73.9 \sim 77.5 \text{ cm}^{-1}$ (308~314)。分析同一品种玉米种子在0~1 d和1~2 d老化期间筛选得到胚乳和种胚特征谱区可得,共同区间较少,差异谱区较多,表明种胚和胚乳老化过程中的变化是不同的,可能是由于种胚和胚乳所含成分差异较为显著,导致老

宽度为10,阈值为0.3。以中地77玉米种子为例,人工老化0~1 d的胚乳ATR吸收谱的移动窗口相关系数如图6所示。

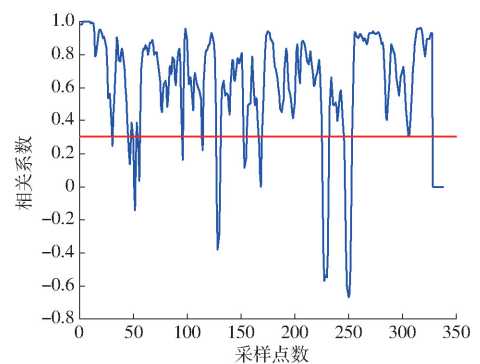


图6 中地77玉米种子胚乳老化0~1 d移动窗口相关系数

Fig. 6 Moving window correlation coefficient diagram of Zhongdi 77 corn seeds endosperm between aging 0 d and 1 d

3个品种的玉米样本在不同老化阶段之间的移动窗口系数特征波段可视化示意图如图7所示。

化过程中发生的生理生态变化也有所不同,但是谱区对应的光谱信息如何映射种子老化或是活力变化还有待解析。

## 3 结束语

实验采集了3个不同品种的玉米种子在老化过程中的太赫兹衰减全反射光谱数据,利用移动窗口相关系数法搜索种子在不同老化阶段的特征谱区。实验结果表明,太赫兹光谱技术结合移动窗口相关系数法对玉米种子老化过程变化的动态监测是可行的,可为玉米种子老化过程中的活力监测提供一种可行的方案。

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(上接第386页)

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